THE ACUTE EFFECTS OF A SINGLE SESSION OF PLYOMETRIC EXERCISE ON MARKERS OF BONE TURNOVER IN BOYS AND MEN

by

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Submitted in partial fulfillment of the requirements for the degree of

Master of Science in Applied Health Sciences

(Kinesiology)

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ABSTRACT

The study objective was to compare the response of bone markers to an exercise session consisting of high mechanical loading (144 jumps) between boys (n=12, 10.2 ± 0.4 years) and men (n=18, 22.5 ± 0.7 years). Blood samples were collected at pre-, 5, 60 minutes post-, and 24 hours post-exercise) to measure bone-specific alkaline phosphatase (BAP), amino-terminal cross-linking telopeptide (NTx), osteoprotegrin (OPG) and receptor activator of nuclear factor kb ligand (RANKL). Boys had higher BAP levels at all time points, with an increase 24 hours post-exercise. No such increase was observed in men. Likewise, NTx levels were higher in boys, with a greater increase over time than in men. OPG and RANKL levels were similar in boys and men at all times. In summary, even one session of exercise stimulates bone turnover, as reflected in the increase in both BAP and NTx, in boys (but not men) within 24 hours.

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ABBREVIATIONS

ANOVA	Analysis of variance
AP	Alkaline phosphatase
BAP	Bone-specific alkaline phosphatase
BIA	Bioelectrical impedance analysis
BMC	Bone mineral content
BMD	Bone mineral density
BMI	Body mass index
BPAQ	Bone-specific physical activity questionnaire
bpm	Beats per minute
CON	Control
CPM	Counts per minute
CTx	C-telopeptides of type I collagen
CV	Coefficient of variation
DEXA	Dual-energy x-ray absorptiometry
Dpd	Deoxypyridinonine
ELISA	Enzyme-linked immunosorbent assay
F	Formation
FFM	Fat free mass
G xT	Group-by-Time
GH	Growth hormone
GRF	Ground reaction forces
HR	Heart rate
ICTP	Carboxy-terminal telopeptide of type I collagen
IGF-I	Insulin-like growth factor 1

IR	Intermittent running
LBM	Lean body mass
LTPA	Leisure time physical activity
nM BCE	Nanomoles bone collagen equivalents
NTx	N-telopeptides of type I collagen
OC	Osteocalcin
OPG	Osteoprotegerin
PA	Physical activity
PBF	Percent body fat
PH	Tanner pubic hair
PHV	Peak height velocity
PICP	Procollagen type I c-terminal peptide
PINP	Procollagen type I n-terminal peptide
PL	Plyometrics
pQCT	Peripheral quantitative computed tomography
PTH	Parathyroid hormone
PYPAQ	Past year physical activity questionnaire
Pyr	Pyridinoline
QCT	Quantitative computed tomography
QUS	Quantitative ultrasound
R	Resorption
RA	Recreationally active
RANK	Receptor activator of nuclear factor κB
RANKL	Receptor activator of nuclear factor κB ligand
RE	Resistance exercise

RM ANOVA	Repeated measures analysis of variance
RM	Repetition maximum
SOS	Speed of sound
T	Trained
TRAP5b	Plasma tartrate-resistant acid phosphatase
UT	Untrained
WBV	Whole body vibrations

Chapter 1: Introduction

1.1 Rationale

Physical activity (PA) plays a vital role in the development and maintenance of skeletal mass throughout the life span, as shown in several meta-analyses (Behringer, Gruetzner, McCourt, & Mester, 2013; Marques, Mota, & Carvalho, 2012; Wolff, van Croonenborg, Kemper, Kostense, & Twisk, 1999). The positive effects of PA can vary throughout life's different stages, where the most evident benefits can be seen during prepubescent and pubescent skeletal growth and development (K. B. Gunter, Almstedt, & Janz, 2012). During childhood and adolescence, high impact exercise, such as jumping, appears to have the greatest benefits on bone structure and mineralization. There are several impact exercise intervention studies, such as jumping, in prepubescent, pubescent and young adult populations which demonstrate the beneficial effect of high impact exercise training on bones, in terms of the changes in the amount of bone mineral over time (bone mineral accrual) and bone structure (Fuchs, Bauer, & Snow, 2001; K. Gunter, Baxter-Jones, Mirwald, Almstedt, Fuchs et al., 2008; K. Gunter, Baxter-Jones, Mirwald, Almstedt, Fuller et al., 2008; Janz et al., 2004; Mackelvie, McKay, Khan, & Crocker, 2001; MacKelvie, McKay, Petit, Moran, & Khan, 2002; MacKelvie, Khan, Petit, Janssen, & McKay, 2003; MacKelvie, Petit, Khan, Beck, & McKay, 2004; Petit et al., 2002; Warden & Fuchs, 2009; Weeks, Young, & Beck, 2008). In terms of bone mineral density (BMD) in children it has been shown that non-impact sports, such as swimming, results in lower BMD in comparison to high impact sports, such as gymnastics (Cassell, Benedict, & Specker, 1996; Courteix et al., 1998). Therefore, not only is the timing of

physical activity a very important determining factor but also the type of physical activity (K. B. Gunter et al., 2012).

While there is a clear correlation between physical activity and either increased BMD in children and adolescents or prevention of bone loss in adults, the particular mechanism(s) responsible for the increase or maintenance in BMD are not well understood. More specifically, it is not clear how physical activity affects bone cell activity that, in time, results in differences in BMC and BMD. The use of biochemical measurements of bone turnover provides information on bone cell activity, specifically bone formation by the osteoblast and bone resorption by the osteoclast. The use of markers of bone turnover allows for an estimation of the bone metabolic processes and any changes in bone turnover.

While PA is a known contributor to bone turnover, the response of bone markers to exercise depends on the magnitude, intensity and duration of exercise. Short- and long-term training appears to have beneficial effects on markers of bone formation and bone resorption (Banfi, Lombardi, Colombini, & Lippi, 2010). According to these studies, there appears to be a pattern of increased levels of markers of bone formation and decreased levels or stabilization of markers of bone resorption as a response to exercise training in adults and adolescents but there is limited research in children. Most studies which examined the effect of training on bone turnover involve a protocol in which blood sampling takes place 24-48 hours following the last exercise session. However, it is possible that this protocol precludes the detection of an acute exercise effect.

Studies that examined the acute effects of exercise on markers of bone turnover in adults, have used various forms of exercise and blood sample timings and are therefore,

highly inconsistent (Banfi et al., 2010). Resistance training typically resulted in decreased bone resorption markers and unchanged bone formation markers, whereas endurance running typically resulted in increased markers of both bone formation and bone resorption (Ashizawa et al., 1998; Brahm, Piehl-Aulin, & Ljunghall, 1997; Langberg, Skovgaard, Asp, & Kjaer, 2000; Lippi et al., 2008; Malm, Ronni-Sivula, Viinikka, & Ylikorkala, 1993; Nishiyama, Tomoeda, Ohta, Higuchi, & Matsuda, 1988; R. S. Rogers, Dawson, Wang, Thyfault, & Hinton, 2011; Scott et al., 2010; Scott et al., 2011; Scott et al., 2013; Thorsen, Kristoffersson, Hultdin, & Lorentzon, 1997; Welsh et al., 1997; Whipple et al., 2004; Ziegler et al., 2005). Non-weight bearing exercise, such as cycling, and plyometric exercise do not appear to have a clear pattern or trend (Guillemant, Accarie, Peres, & Guillemant, 2004; Herrmann et al., 2007; Kristoffersson, Hultdin, Holmlund, Thorsen, & Lorentzon, 1995; Lin et al., 2012; Pomerants et al., 2008; R. S. Rogers et al., 2011; Rong et al., 1997; Rudberg, Magnusson, Larsson, & Joborn, 2000; Wallace et al., 2000). In children, there is one study, by Pomerants et al. (2008), who examined the acute effects of 60 minutes of high intensity cycling on markers of bone turnover in boys of different pubertal stages. The authors reported no changes in markers of bone metabolism 30 minutes following the cycling bout (Pomerants, Tillmann, Karelson, Jurimae, & Jurimae, 2008).

Therefore, the purpose of this study was to compare the acute response and recovery of bone metabolism induced by a high-impact, plyometric exercise protocol over a period of 24 hours between pre-pubertal children and adults. This study will help to standardize the timing of sampling in terms of when the effects of exercise occur and whether or not the effect lasts longer than 24 hours. From a methodological perspective, this

information will aid researchers in determining the appropriate timing to examine the effects of an intervention on bone metabolism, without contamination of a prior exercise session. Aside from the methodological purpose, a more practical aspect involves the determination of whether bone markers measurement in serum is sensitive enough to detect changes in bone metabolism following a single bout of exercise, as well as provide insight into the timeline of these changes. This is important information as it can elucidate the early phase of bone response to mechanical loading.

1.2 Objectives and Hypotheses

The objective of this study was three-fold. First, to determine the acute effects of a single bout of impact exercise on bone formation markers over 24 hours in both boys and men. The second objective was to determine the acute effects of a single bout of impact exercise on bone resorption markers over 24 hours in both boys and men. The third objective was to determine whether or not there was a difference in the effect of exercise on bone markers between boys and men.

It was hypothesized that: (1) markers of bone formation would increase 1 hour post exercise and subsequently decrease 24 hours post exercise, (2) markers of bone resorption would increase 5 minutes post exercise and subsequently decrease to baseline values 24 hours post exercise and (3) children would have higher values for both markers of bone formation and resorption in comparison to the adults but both groups will follow the same trend with respect to the timing of the alterations.

Chapter 2: Review of Literature

2.1 Bone

2.1.1 Bone Anatomy, Development and Turnover

The human skeleton contains 206 individual bones that provide the body with a multitude of important functions: support for the body, protection for vital structures, mineral reservoir composed mainly of calcium and phosphatase, and the mechanical basis for muscle movements. Skeletal tissue is predominantly composed of collagen fibers and minerals in the form of hydroxyapatite, derived mostly from calcium and phosphate (Bailey et al. 1996). The skeleton can be divided into the axial skeleton that includes the skull, vertebral column, sternum and ribs and the appendicular skeleton that includes the upper and lower extremities, as well as the shoulder and pelvic girdle (Moore, K.L. and Agur, A.M.R., 2007). Bone tissues can be mineralized into two forms: (1) cortical bone and (2) trabecular bone. Approximately 75-80% of the total skeletal mass is comprised of cortical bone and the remaining 20-25% of the mass is trabecular bone (Moore, K.L. and Agur, A.M.R., 2007). Cortical bone forms the outer surface of all bones, including the diaphysis of long bones, and is densely compacted tissue. Trabecular bone is comprised of a meshwork of thin, boney horizontal and vertical plates giving it a porous structure and is located on the inside of the cortical shell of flat bones, vertebral bodies and the epiphysis of long bones (Moore, K.L. and Agur, A.M.R., 2007). Because trabecular bone has a relatively larger surface area to volume ratio than cortical bone, it is suggested that this form of bone is more susceptible to hormonal changes and that it also has a higher

turnover rate. Cortical bone alternatively provides more protective, biomechanical and supportive properties (Moore, K.L. and Agur, A.M.R., 2007).

Bone development is comprised of two very distinct phases; (1) skeletal patterning and (2) mineralization. Skeletal patterning occurs during the embryonic period, when the shape and position of various skeletal components are determined through local growth factors and numerous regulatory gene expressions. The second phase, mineralization, is highly influenced by mechanical stress and strain (Davies, Evans, & Gregory, 2005). Heredity is known to be a major determining factor of bone mineral status although there has been increasing supporting evidence to show that a large portion of the variance in BMD may be attributable to non-hereditary factors such as exercise and nutrition (Bailey et al. 1996).

Bone turnover consists of three simultaneous processes each functioning differently; activation, resorption and formation (Bailey et al. 2006). Activation is the first stage in bone turnover, where pre-osteoclasts are attracted to the modeling sites and fuse to form multinucleated osteoclasts (Bailey, Faulkner, & McKay, 1996). Bone resorption involves the cells that resorb bone, which are known as osteoclasts. Bone formation involves the bone-forming cells found on the surfaces of bone and in bone cavities, which are known as osteoblasts. If the rate of bone formation exceeds that of bone resorption the expectant result is bone mineral accrual, as seen during the growth years. In fact, during the adolescent growth spurt approximately half of the peak bone mineral content is attained (Malina et al. 2004; Seibel et al. 2002). Therefore, childhood through to late adolescence is considered a critical period of bone accrual (Seibel, 2002).

Once skeletal maturity is reached, bone tissue continues the process of constant renewal of old bone and its replacement by new bone, through coordinated activity of osteoblasts and osteoclasts. Bone turnover allows the body to respond to external mechanical forces and molecular signals, in addition to providing a mechanism for the maintenance of calcium homeostasis (Calvo, Eyre, & Gundberg, 1996). Osteoclasts initiate bone turnover by eroding mineralized surfaces, forming a cavity where osteoblasts can deposit new bone (Calvo et al., 1996). During steady state, the "coupling" of bone formation and bone resorption maintains bone mass but if bone resorption exceeds bone formation this may result in debilitating bone diseases such as osteoporosis (Calvo et al., 1996).

The age of the onset of the accelerated rate of bone mineral accrual and the age of attainment of peak bone mineral content may vary between sexes (Bailey et al. 1996). In childhood, bone mineral content increases linearly with age, with no evident sex differences until puberty. However, during the course of growth and maturation, females undergo their growth spurt prior to males and as a result have a slightly higher bone mineral content during early adolescence. Since males experience their growth spurt later on, their bone mineral content continues to increase in late adolescence. Bone mineral content continues to increase in males throughout and into the third decade, whereas the total-body bone mineral content reaches a plateau, with only small increases at approximately 20 years of age in females. The sex differences will thus be established in late adolescence (Bailey et al., 1996; Malina, R.M., Bouchard, C. & Bar-Or, O., 2004; Theintz et al., 1992).

2.2 Bone Assessment Techniques

The state of the skeleton and any changes in bone mineralization can be evaluated through several different techniques. Typically, dual-energy x-ray absorptiometry (DEXA) is used to measure bone mineral content from childhood right through to adulthood as it is quick, precise and the amount of radiation exposure is generally viewed as acceptable. This method provides an estimate of areal bone mineral density (g/cm^2) acquired from the ratio of bone mineral content to bone area (Malina, R.M., Bouchard, C. & Bar-Or, O., 2004). BMD assessed through DEXA is only considered an estimation of 'true' bone density as this method is measured in two dimensions and a true volumetric density is measured 3-dimensionally (Fulkerson et al., 2004). BMD measured by DEXA may not properly reflect true BMD as DEXA does not take into account the depth of the bones.

For growing children, the preferred method of use for research purposes is often quantitative computed tomography (QCT) or peripheral QCT (pQCT), which provides a true measurement of volumetric bone mineral density. It also provides measures of bone size and geometry, both of which can affect bone strength. However, like DEXA, there is exposure to radiation, and the levels of exposure are higher than with DEXA and in a more focused region (Schoenau et al., 2004). QCT is also relatively expensive and does not provide a reference database for children.

Quantitative ultrasound (QUS) has recently been developed for the assessment of bone strength and fracture risk in cortical bone. The advantage of transaxial QUS is that it measures the speed of sound (SOS), reflecting bone properties without being influenced

by bone size (Barkmann et al., 2000; Schoenau et al., 2004). QUS is also relatively inexpensive, non-invasive, radiation-free and portable. SOS can be measured either transversally, at trabecular bone sites or transaxially, along cortical bone. Transaxial assessments are limited to peripheral sites of the body, such as the radius and tibia, as the method involves transmission of sound waves along the length of the bone. The SOS values are a reflection of not only bone mineral but also structure and elasticity (Barkmann et al., 2000; Njeh et al., 1999; Schoenau et al., 2004).

2.3 Biochemical Markers of Bone Turnover

Because the previously mentioned bone assessment techniques do not allow for dynamic bone examination or are slow to reveal any intervention-induced changes, biochemical markers of bone turnover are useful for measuring acute changes in bone cell activity (Calvo et al., 1996; Watts, 1999). Biochemical markers of bone turnover can be measured via blood or urine and can be divided into two categories; (1) enzymes or proteins involved in bone formation (generated by osteoblasts) and resorption (generated by osteoclasts) and (2) the formation and degradation products of bone matrix metabolism (primarily type I collagen (Figure 1.) (Calvo et al., 1996; Watts, 1999). A limitation of these markers is that systemic biochemical markers cannot be narrowed to specific skeletal sites (Calvo et al., 1996; Christenson, 1997; Watts, 1999).

2.3.1 Markers of Bone Formation

The traditional markers of bone formation are either direct or indirect products of osteoblast activity and include one enzyme, bone-specific alkaline phosphatase and three byproducts of bone matrix synthesis, osteocalcin and carboxy and amino procollagen

extension peptides; all of which can be measured in the blood (Figure 1.) (Banfi et al., 2010; Calvo et al., 1996; Watts, 1999).

2.3.1.a Bone-Specific Alkaline Phosphatase (BAP)

Alkaline phosphatase (AP) is an enzyme associated with plasma membranes of bone, liver, intestines and placenta, all of which can contribute to the amount of AP found in the blood (Banfi et al., 2010; Calvo et al., 1996; Watts, 1999). Bone-specific alkaline phosphatase (BAP) is an enzyme localized to the membrane of osteoblasts and plays an important role in bone mineralization thereby providing a specific indicator for osteoblast activity (van Straalen, Sanders, Prummel, & Sanders, 1991). BAP has been used as a diagnostic marker in various bone pathologies such as osteomalacia, rickets, Paget's disease and for monitoring the healing of new fractures (Calvo et al., 1996; Watts, 1999). In adults, BAP appears to be a more sensitive diagnostic tool when compared to total AP activity, as small increases are detectable and it is tissue-specific (Delmas & Garnero, 1998). BAP is highly predominant throughout childhood, and even more so during and up until mid-puberty and is then followed by a decrease in late puberty or, post menarche for females (Mora, Pitukcheewanont, Kaufman, Nelson, & Gilsanz, 1999; Szulc, Seeman, & Delmas, 2000; van Coeverden et al., 2002). Nutritional intake does not typically influence BAP levels and the intra-individual variability over time is comparatively low (Seibel, 2002).

2.3.1.b Osteocalcin (OC)

Osteocalcin (OC) or GLA protein is a small protein that is synthesized by mature osteoblasts, odontoblasts and hypertrophic chondrocytes and has been studied extensively

as a marker of bone formation (Calvo et al., 1996). An abundance of evidence suggests that OC, the major noncollagen protein of bone matrix, is a sensitive and specific marker for osteoblast activity. The dynamic marker of bone formation is primarily deposited in the extracellular matrix of bone with fragments circulating in the blood (Ivaska et al., 2004; Watts, 1999). Because OC is incorporated into the skeletal matrix and is released during bone resorption it can also be used as a measurement of bone turnover (Ivaska et al., 2004). In growing children, there appears to be a correlation between OC levels, height and height velocity, where OC propeptide levels parallel the height velocity curve. The concentration varies with pubertal level, age and time of day; where the highest levels are observed in the mornings. Nutritional intake does not typically have any influence on OC (Mora et al., 1999; Seibel, 2002; Szulc et al., 2000; van Coeverden et al., 2002).

2.3.1.c Procollagen I Extension Peptides

Procollagen I Extension Peptides, carboxy (PICP) and amino (PINP), constitutes approximately 90-95% of bone collagen with the remaining 5-10% coming from types III and IV. Type I collagen also has a minor component in the extracellular matrix of tissues aside from bone, such as skin, dentin, cornea, vessels and tendons (Banfi et al., 2010; Calvo et al., 1996). PINP and PICP result from the extracellular enzymatic cleavage at the carboxy and amino termini, following the synthesis of new collagen molecules. Because these peptides are generated with collagen synthesis they are considered to be a quantitative reflection of bone formation (Banfi et al., 2010; Seibel, 2002). Although most type I collagen is found in bone, it is not specific for bone and is therefore not the most sensitive index of bone formation (Seibel, 2002; Szulc et al., 2000). Similar to OC,

PICP and PINP are correlated with the growth velocity, peak values occur early in the morning and values are not usually influenced by food intake (Szulc et al., 2000).

2.3.2 Markers of Bone Resorption

The traditional markers of bone resorption typically measure the collagen degradation products from osteoclast activity, with one osteoclast-specific enzyme. Both blood and urine samples can be analyzed, reflecting bone resorption (Figure 1.) (Banfi et al., 2010; Calvo et al., 1996; Seibel, 2002).

2.3.2.a Collagen Pyridinium Crosslinks

Collagen cross-links, pyridinoline (Pyr) and deoxypiridinonine (Dpd), function to cross-link several collagen molecules via molecular bridges, thereby mechanically stabilizing the collagen molecule. They are non-reducible cross-links of bony and cartilaginous collagens that can also be found in the extracellular matrix of other connective tissues. During bone resorption, the cross-linked collagens are broken down with the cross-link components being released into the circulation and urine (Banfi et al., 2010; Seibel, 2002). Pyr is predominantly found in cartilage but can also be found in bone, ligaments and vessels, whereas Dpd is almost exclusively found in bone and dentin. Because bone has a much higher turnover rate in comparison to cartilage, ligaments or tendons, the quantity of Pyr and Dpd found in either the blood or urine can be termed skeleton derived (Seibel, 2002). In children, there is a marked age-related variation in urinary Pyr and Dpd. As with many bone markers Pyr and Dpd correlate with the growth velocity, in that females experience an increase in the early stages of puberty and males experience that increase in the later stages of puberty (Mora et al., 1999; Szulc et al.,

2000). Pyr and Dpd are highest in the morning, lowest in the evening and are not influenced by dietary intake (Watts, 1999).

2.3.2.b Cross-linked Telopeptides

Cross-linked telopeptides of type I collagen are measurements of osteoclast-mediated collagen degradation products that are released with cross-links still attached at both ends. Currently, there are three markers with cross-links still attached; carboxy-terminal collagen cross-links (CTx), amino-terminal cross-linking telopeptide (NTx) and carboxy-terminal telopeptide of collagen type I (ICTP). Both CTx and NTx can be measured in the blood and urine, whereas ICTP can only be measured in the blood (Seibel, 2002; Szulc et al., 2000; Watts, 1999). Similar to the majority of the aforementioned markers of bone turnover the peptide bound resorption markers CTx, NTx and ICTP correlate with growth velocity. Following the growth spurt, urinary and serum levels decrease to the level seen in adults (Marowska et al., 1996). Each telopeptide marker is subject to circadian variations and CTx can be powerfully influenced by food intake, therefore requiring fasting samples (Herrmann & Seibel, 2008; Seibel, 2002; Szulc et al., 2000). Although it has been suggested that most markers are unaffected by nutritional intake, there are no comparable studies that have been published for NTx (Hannon & Eastell, 2000; Herrmann & Seibel, 2008).

2.3.2.c Plasma Tartrate-Resistant Acid Phosphatase (TRAP5b)

Acid phosphatase is comprised of six different isoenzymes, of which only five are resistant to tartrate inhibition, including bone acid phosphatase; also known as tartrate-resistant acid phosphatase (TRAP5b). TRAP5b is synthesized and secreted into the blood

by osteoclasts during bone resorption and is a reflection of the rate of bone resorption (Calvo et al., 1996; Seibel, 2002; Watts, 1999). Unfortunately, TRAP5b does lack specificity for osteoclasts, the presence of enzyme inhibitors in serum and does not provide stable frozen samples (Szulc et al., 2000). Both children, up until menarche in females, and postmenopausal women experience higher levels of TRAP5b. There is very little circadian variation demonstrated and it is not influenced by food intake (Seibel, 2002).

2.3.3 Osteoprotegerin and Receptor Activator of Nuclear Factor κB Ligand Ratio

Osteoprotegrin (OPG), Receptor Activator of Nuclear Factor κB Ligand (RANKL) and Receptor Activator of Nuclear Factor κB (RANK) have been established as the essential cytokines, as new members of the tumor necrosis factor (TNF) ligand and receptor family, for osteoclast life span and as mediators of skeletal diseases (Hofbauer & Heufelder, 2001).

2.3.3.a Osteoprotegerin (OPG)

OPG is considered a key regulator of osteoclastogenesis and is a soluble factor produced by osteoblasts. The effects that OPG has on bone, as shown in vitro, include inhibition of differentiation, survival and fusion of osteoclastic precursor cells, suppression of osteoclast activation and promotion of osteoclast apoptosis (Hofbauer & Heufelder, 2001; Khosla, 2001). Using OPG-deleted knock-out mice, it was established that without this certain molecule severe osteoporosis is developed as there is a marked increase in osteoclast formation, leading to a marked increase in bone resorption (Bucay et al., 1998; Mizuno et al., 1998). The levels of OPG appear to be maintained relatively

stable in children older than the age of 4 years old up until the ages of approximately 40-45 years old, where they typically increase (Khosla, 2001; Wasilewska, Rybi-Szuminska, & Zoch-Zwierz, 2009). Buzi et al.(2004) and Wasilewska et al. (2009) both showed higher values in children between the ages of 1 and 4 years old (Buzi et al., 2004; Wasilewska et al., 2009).

2.3.3.b Receptor Activator of Nuclear Factor κB Ligand (RANKL)

Receptor Activator of Nuclear Factor κB Ligand (RANKL) is well known for its activity on the immune system. With respect to bone metabolism, it has been established that RANKL is a pre-resorptive factor, acting to stimulate osteoclast differentiation, activity and inhibition of osteoclast apoptosis (Hofbauer & Heufelder, 2001; Khosla, 2001). Kong et al. (1999) has demonstrated through RANKL knock-out mice that without the presence of RANKL the end result is severe osteopetrosis and a complete absence of osteoclasts (Kong et al., 1999). On the other hand, when mice were parenterally administered RANKL, a marked enhancement in the generation and activation of osteoclasts was present. Because of this enhancement, the mice demonstrated increases in osteoclast formation and activation, a marked decrease in bone mass resulting in osteoporosis and life threatening hypercalcemia (Lacey et al., 1998). Wasilewska et al. (2009) found differences in serum concentration of RANKL between sexes and among age groups. The level of RANKL was much higher in males when compared to females. This finding was also reported in adults by Kerschan-Schindl et al. (2008). A statistically significant difference in children younger than 9 years old versus children older than 9 years old was also apparent with the older children having higher levels (Wasilewska et al. 2009). However, the age correlation in children was not apparent in other studies

(Kerschan-Schindl et al., 2008; Wasilewska et al., 2009). An age-dependent reduction of serum RANKL levels has been suggested in post-menopausal women, which may help to counteract the reduction in bone formation for the elderly population (Liu et al., 2005).

2.3.3.c Receptor Activator of Nuclear Factor κB (RANK)

Receptor Activator of Nuclear Factor κB (RANK) is the receptor for RANKL. The functional expression of RANK is found mainly in osteoclasts and dendritic cells. It was demonstrated in RANK knock-out mice that RANK was the exclusive receptor and was expressed on preosteoblastic cells as the mice experienced severe osteopetrosis due to the absence of osteoclasts, similar to the RANKL knock-out mice (Hofbauer & Heufelder, 2001; Khosla, 2001; Liu et al., 2005).

2.3.3.d OPG/RANKL/RANK

Osteoblasts and stem cells within the bone marrow produce RANKL, which is able to bind with a transmembranic receptor, RANK, which is found on preosteoclastic cells. The interaction between RANKL and RANK results in the activation of the nuclear factor κ B, which helps to promote the formation of preosteoclasts into active osteoclasts. If however, there is a reduction in RANKL the result is the inactivation of its receptor, RANK, and bone resorption is reduced. RANKL is a very strong activator of osteoclasts but can be counteracted by OPG, the protector cytokine of bone. OPG competitively binds to RANKL, which reduces the impact that RANKL has on its receptor, RANK and preosteoclasts. In doing so, a reduction of the transformation of preosteoclasts to osteoclasts is achieved which enhances the formation of bone. The importance of the ratio between OPG and RANKL then becomes highly apparent. It has been suggested

that OPG and RANKL play an essential role in a variety of metabolic bone diseases such as, osteoporosis, rheumatoid arthritis, Paget's disease and periodontal disease (Figure 1.) (Khosla, 2001; Ziegler et al., 2005).

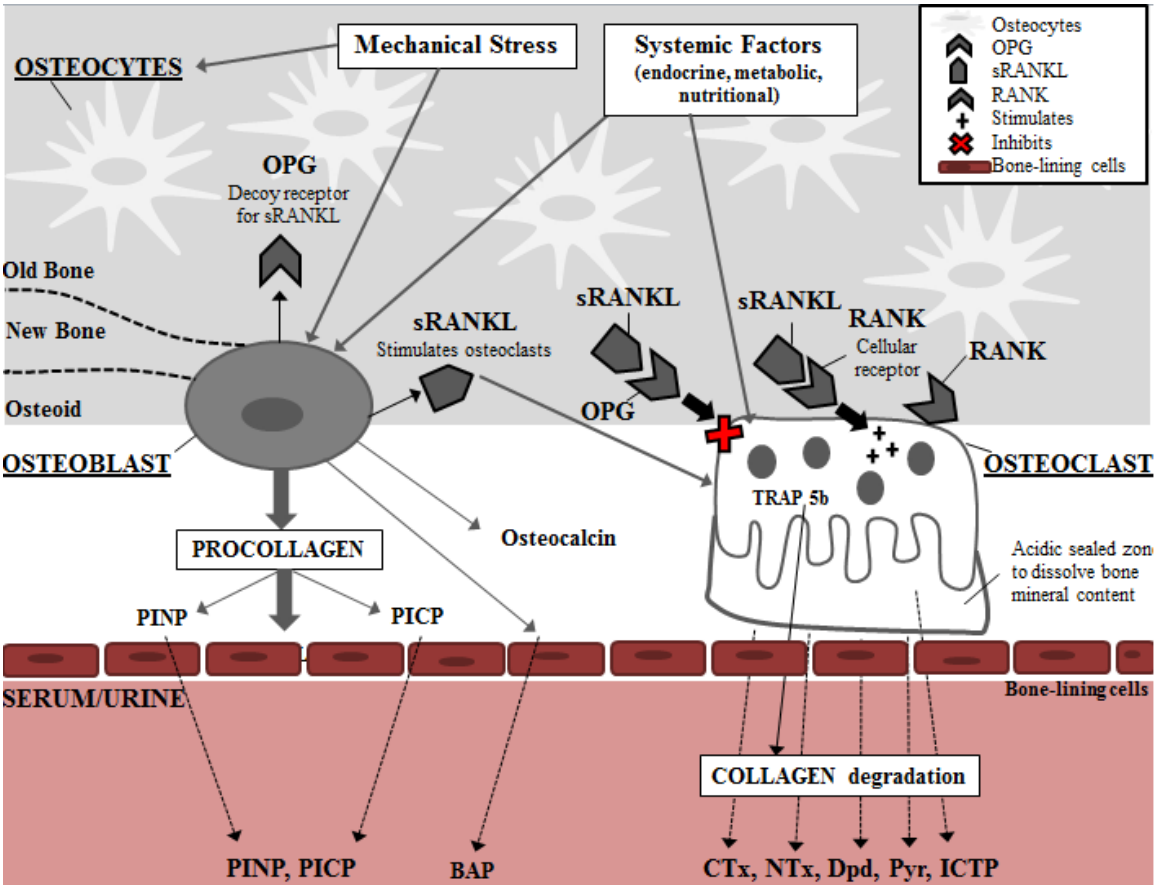


Figure 1. Bone Turnover. Bone turnover is an ongoing dynamic process that consists of bone resorption, achieved by **osteoclasts**, and bone formation, achieved by **osteoblasts**. The initiation of bone turnover can take several forms, direct mechanical stress on the bone or systemic changes in homeostasis (*e.g.* endocrine, metabolic, nutritional). **Osteoclasts** are responsible for the resorption of the bone matrix (**osteoid**) which results in collagen degradation products such as tartrate resistant isoenzyme of acid phosphatase (**TRAP 5b**), pyridinoline (**Pyr**), deoxypiridinonine (**Dpd**), carboxy-terminal collagen cross-links (**CTx**), amino-terminal collagen cross-links (**NTx**) and carboxy-terminal telopeptide of collagen type I (**ICTP**). **Osteoblasts** are responsible for the formation and organization of the extracellular matrix of bone and its subsequent mineralization, as well as the synthesis of collagen and other bone proteins. The direct or indirect products of this osteoblastic activity can be classified as bone-specific alkaline phosphatase (**BAP**), **Osteocalcin** and procollagen I Extension Peptides, carboxy (**PICP**) and amino (**PINP**). As osteoblasts form new bone tissue many become embedded within the matrix and differentiate into **osteoctytes**. Osteoprotegerin (**OPG**), secreted by many cell types, including osteoblasts, is the decoy receptor that inhibits the differentiation and activation of osteoclasts. Soluble receptor activator of nuclear factor κB ligand (**sRANKL)** also secreted by osteoblasts, is a main stimulator for the formation of mature osteoclasts. Receptor activator of nuclear factor κB (**RANK**), expressed by mature osteoclasts, is the

membrane receptor for **RANKL**. The binding interaction between **RANKL** and **RANK** promotes the formation of preosteoclasts into active osteoclasts.
(Datta, Ng, Walker, Tuck, & Varanasi, 2008; Kular, Tickner, Chim, & Xu, 2012; Raggatt & Partridge, 2010; Teco Medical Group, 2009)

2.4 Biological Variability

Biochemical markers of bone turnover are used as indirect indices of skeletal metabolism. The analyses of biochemical bone markers need to be standardized and stable to account for any biological variability. There are a number of contributing sources to biological variability and they can be divided into two categories: (1) uncontrollable factors, and (2) controllable factors (Hannon & Eastell, 2000). The uncontrollable factors include age, sex, menopausal status, menstrual cycle, pregnancy, ethnicity, fractures, seasonal, disease, bed rest or immobility, circadian rhythm and hormones. These factors cannot be altered, but by using age-appropriate and sex-specific reference ranges alongside with recent published information regarding fractures and diseases, they can be accounted for. The controllable factors include drugs, oral contraception, diet and exercise, and if standardization and appropriate timing and conditions of the samples are undergone then these factors can be manipulated to reduce the effects of variability (Hannon & Eastell, 2000).

2.4.1 Uncontrollable Factors

2.4.1.a Age, Sex, Puberty

Age can affect biochemical markers of bone turnover, specifically at three stages of the life cycle: infancy, puberty and for women, menopause. At birth, all markers of bone formation and resorption are high and it is not until between the third month and third year of life that they decrease, in correspondence to the slowing of linear growth

(Szulc et al., 2000). Bone markers then remain at constant levels until the initiation of puberty, at which time major fluctuations occur, in accordance with the growth velocity or changes in secondary sex characteristics. In terms of chronological age, females reach their peak in bone turnover markers at an approximate age of 12 and boys at an age of 14. Because the growth velocity and onset of puberty takes place at an earlier age in females then it does in males, the peak bone mineral density is obtained earlier in females. Following the period of accelerated growth, the biochemical bone markers undergo a decrease in both males and females (Hannon & Eastell, 2000; Szulc et al., 2000).

Once women reach menopause, there is a marked increase in bone turnover markers, with the increase in bone resorption markers being greater than the increases in bone formation markers. These markers generally remain elevated throughout the aging process. In men, most bone turnover markers do not change until the fifth or sixth decade is reached, at which time both resorption and formation markers reach their lowest levels with no subsequent change (Hannon & Eastell, 2000).

Premature infants and low birth-weight infants may suffer the adverse effects of having low markers of bone formation (Mora, Weber, Bellini, Bianchi, & Chiumello, 1994; Namgung, Tsang, Specker, Sierra, & Ho, 1993). This reduction may in part be due to reduced vitamin D, vitamin K, insulin-like growth factor 1 (IGF-I) or growth hormone (GH) biological activity (Namgung et al., 1993). An increase in bone resorption markers may also be present in preterm infants. In most cases children catch up with regards to body size but skeletal maturation and the attainment of peak BMD may be delayed and GH therapy may sometimes be implemented (Namgung et al., 1993; Szulc et al., 2000).

2.4.1.b Menstruation

The menstrual cycle induces very minor, if any, changes to levels of bone turnover markers (Chiu et al., 1999). During the luteal phase, markers of bone formation appear to be elevated in comparison to the follicular phase, where both BAP and OC reach the highest levels in the mid-luteal phase and PICP reaching the highest levels in the early luteal phase (Nielsen, Brixen, Bouillon, & Mosekilde, 1990). On the other hand, changes in the levels of bone resorption markers throughout the menstrual cycle are inconsistent. Because the changes are considered small the effect of the menstrual cycle on markers of bone turnover can be termed insignificant (Hannon & Eastell, 2000).

2.4.1.c Pregnancy

Pregnancy and lactation result in a growing calcium demand for the fetus or infant in part provided by an increase in all but one bone turnover markers. OC shows a significant decrease, almost to the point of being undetectable, during pregnancy and this may be due to the placental clearance of OC. Once lactation is discontinued bone turnover markers should return to premenopausal levels (Naylor, Iqbal, Fledelius, Fraser, & Eastell, 2000).

2.4.1.d Ethnicity and Race

Ethnic differences have been reported between black and white populations, with a trend for markers of bone resorption (TRAP5b and Pyr) and formation (OC) to be lower in black children and young adults suggesting that bone turnover was slower (Pratt, Manatunga, & Peacock, 1996). These differences may elucidate why black populations

have a higher bone mineral density and lower fracture incidence (Hannon & Eastell, 2000).

2.4.1.e Fractures

During the healing process of a fracture, markers of bone formation and resorption are significantly increased. This increase may begin as early as the first or second week after the fracture and can continue up until one year after. These factors should be taken into account when analyzing and interpreting bone markers (Hannon & Eastell, 2000; Ingle, Hay, Bottjer, & Eastell, 1999).

2.4.1.f Disease

Metabolic bone diseases are the primary, but not only, pathologies that can impact biochemical markers of bone turnover. Paget's disease is a reflection of increased bone formation markers, BAP and OC. Other diseases such as diabetes and thyroid disease can also alter the levels of bone markers (Hannon & Eastell, 2000). For instance, hyperparathyroidism can cause an increase of up to 3- fold the normal level or remain unchanged in markers of bone resorption. Similarly, markers of bone formation can increase up to 2- to 3-fold the normal level or also remain unchanged (Cortet et al., 2000). The bone marker alterations in nonskeletal diseases may imply metabolic disturbances or extraskeletal production (Delmas et al. 2000).

For those children who suffer from a GH deficiency, stunted skeletal maturation and stature is often present. The bone mass accumulation is typically slower and this may be in part due to the suppression of bone turnover. Markers of bone formation (BAP, OC and PICP) appear to be either lower or normal and markers of bone resorption (Pyr, Dpd

and ICTP) also appear to be lower. GH treatment stimulates the activation of bone turnover resulting in an increase in all biochemical markers of bone formation and resorption, BMC and height (Kubo, Tanaka, Inoue, Kanzaki, & Seino, 1995; Szulc et al., 2000).

2.4.1.g Bed Rest and Immobility

The consequence of bed rest or immobility is a very rapid increase in bone resorption markers. Several cross-sectional studies have revealed that after only 2 days of bed rest Pyr and Dpd significantly increase, with an additional 30-50% increase by one week (van der Wiel, Lips, Nauta, Netelenbos, & Hazenberg, 1991; Zerwekh, Ruml, Gottschalk, & Pak, 1998). Bone formation markers remain unchanged and once remobilization takes place the markers of bone resorption gradually decline to normal levels (Hannon & Eastell, 2000).

2.4.1.h Seasonal

Seasonal changes have been suggested to have low effect on the markers of bone turnover, with a minor difference apparent between the winter and summer months (Woitge, Scheidt-Nave et al., 1998). Most markers of bone resorption are elevated during the winter, with the exception of Pyr excretion being elevated in the summer (Douglas et al., 1996). BAP levels appear to be decreased in the winter and spring, whereas OC may be elevated during those seasons; the results are inconsistent (Douglas et al., 1996). The seasonal variations are small but may be explained by the vitamin D deficiency often seen throughout the winter months, but in areas of low latitude this may be less marked as exposure to sunlight tends to be higher (Hannon & Eastell, 2000; McKenna, 1992).

2.4.1.i Circadian Rhythm

The impact of circadian variability is greater than most other contributing factors of variability. Both urinary and serum markers of bone turnover have significant circadian rhythms. Most markers experience high fluctuations at night/early morning and rapidly decline to reach a nadir between 1300 and 2300hrs, although each marker appears to be different. It is also important to note that nightly calcium supplementation and bisphosphonate treatment can suppress both the circadian rhythm and bone resorption markers. Circadian rhythms and the factors that can affect it can be very substantial and precise timing of sample collection is of utmost importance for accurate interpretation of the results as outlined in table 1. (Delmas et al., 2000; Hannon & Eastell, 2000). The rhythms of OPG and RANKL have not been well-studied and the results appear to be inconsistent. Both Dovio et al. (2008) and Ohta et al. (2006) demonstrated that neither OPG nor RANKL have a significant circadian rhythm in premenopausal women whereas Joseph et al. (2006) did find a rhythmicity in OPG in women between the ages of 25 and 65 years (Dovio et al., 2008).

Table 1. Peak and nadir timing for markers of bone formation and bone resorption.

Reference	Bone Marker	Medium	Peak	Nadir
Hassager et al., 1992 ; Schlemmer et al., 1992	Pyr	Urine	0200hrs – 0800hrs	1400hrs – 2300hrs
	Dpd	Urine	0200hrs – 0800hrs	1400hrs – 2300hrs
Aoshima et al., 1998; Wichers et al., 1999	CTX	Serum	0130hrs – 0430hrs	1100hrs – 1500hrs
	NTX	Serum	0130hrs – 0430hrs	1100hrs – 1500hrs
Greendspan et al., 1997	BAP	Serum	1140hrs – 1245hrs	0630hrs
Gundberg et al., 1985	OC	Serum	0203hrs – 0400hrs	1200hrs – 1600hrs
Hassager et al., 1992	PICP	Serum	0200hrs – 0500hrs	~1400hrs
	PINP	Serum	0200hrs – 0500hrs	~1400hrs

(Aoshima et al., 1998; Greenspan, Dresner-Pollak, Parker, London, & Ferguson, 1997; Gundberg, Markowitz, Mizruchi, & Rosen, 1985; Hassager et al., 1992; Hassager, Risteli, Risteli, Jensen, & Christiansen, 1992; Schlemmer, Hassager, Jensen, & Christiansen, 1992)

2.4.1.j Hormones

Certain hormones can play an essential role in the maintenance of skeletal health throughout one's lifespan. Hormone deficiencies can occur in both sexes throughout childhood or adulthood, thereby having potentially adverse effects on children and adults. The main hormones involved in the maintenance of bone health include growth hormone (GH), parathyroid hormone (PTH), testosterone, estrogen and IGF-1. These hormones and growth-factors also experience fluctuations throughout the day and the peak times and nadir times need to be taken into consideration for the timing of samples so to avoid any impact on the analyzed results (Compston, JE. 2001). To reduce the effect of

hormonal fluctuations it is essential that the timing of sample collection is tightly controlled outside of the peak and nadir timings.The various timing is outlined in Table 2.

Table 2. Peak and nadir timing of bone-related hormones and growth factors

Reference	Hormone/ Growth-factor	Sex	Medium	Peak	Nadir
Surya et al., 2006	GH	M	Plasma	2400hrs*, 1200hrs & 1800hrs	0800hrs (waking hours)
		F		2400hrs*, 1100hrs & 1600hrs	
Jubiz et al., 1972	PTH	M/F	Serum	0200hrs – 0400hrs	0800hrs
Ahokoski et al., 1998; Plymate et al., 1989	Testosterone	M	Serum	0800hrs (early morning hours)	1600hrs – 2000hrs
Ahokoski et al, 1998;	Estrogen	M	Serum	1200hrs	2000hrs
Heuck et al., 1999	IGF-1	M/F	Serum	0900hrs & 1200hrs	0700hrs

*Signifies the maximum occurrence

(Ahokoski et al., 1998; Heuck, Skjaerbaek, Orskov, & Wolthers, 1999; Jubiz, Canterbury, Reiss, & Tyler, 1972; Plymate, Tenover, & Bremner, 1989; Surya, Symons, Rothman, & Barkan, 2006)

2.4.2 Controllable Factors

2.4.2.a Drugs

The main forms of pharmaceuticals that can have a direct impact on biochemical bone turnover are antiresorptive drugs. These drugs are typically used for the treatment of osteoporosis but there are also other medicinal treatment methods for various metabolic bone diseases. Bisphosphonates are widely known to promptly reduce markers of bone turnover by up to 70% (Hannon & Eastell, 2000). Oral corticosteroid use can reduce OC

and PICP by up to 40-50% after a few days (Oikarinen et al., 1992). Anticonvulsant medication can also affect markers of bone turnover. Large increases in Pyr and Dpd and moderate increases in markers of bone formation have been shown following a long-term treatment plan of anticonvulsant medication (Ohishi et al., 1996). Similarly, GnRH agonist treatment has been shown to increase bone turnover significantly, whereas the use of thiazide diuretics can significantly decrease markers of bone turnover (Delmas et al. 2000). Rheumatoid arthritis often requires the use of long-term corticosteroid therapy, which acts to subdue bone formation. The use of corticosteroids in children, often used for the treatment of asthma, not only reduces bone turnover but also growth and BMD (Boot, de Jongste, Verberne, Pols, & de Muinck Keizer-Schrama, 1997). Corticosteroids act to inhibit bone resorption which is shown through the reduced levels of Pyr, Dpd and ICTP. Corticosteroids also have a direct suppressive effect on osteoblasts and because bone resorption precedes bone formation, this may also impact bone formation markers (Sorva, Turpeinen, Juntunen-Backman, Karonen, & Sorva, 1992).

2.4.2.b Oral Contraceptives

The effect of oral contraceptives is not entirely known but is suggested to be age-dependent. Significant decreases in markers of bone formation and resorption have been shown in women between the ages of 35 and 49, which is not the typical age group for oral contraceptive consumption (Garnero, Sornay-Rendu, & Delmas, 1995). However, there is little to no effect in younger women (Hannon & Eastell, 2000).

2.4.2.c Dietary Intake

Dietary intake does not typically affect markers of bone turnover, aside from serum CTx (Seibel, 2002). As aforementioned, CTx is strongly influenced by food intake and to ensure accuracy an overnight fasting sample should be implemented (Seibel, 2002). Mineral dietary supplementation, such as zinc, copper or magnesium, does seem to impact markers of bone turnover (Hannon & Eastell, 2000). Calcium supplementation can suppress markers of bone turnover, especially in pre- and post-menopausal women. This may depend on the time of day the supplement is taken (Ginty, Flynn, & Cashman, 1998). Long-term vitamin D supplementation, of either 5 or 10μg/day has been shown to decrease bone resorption markers, while having no effect on bone formation markers (Rossini et al., 2012). The short-term effects of a high dose of vitamin D (600 000 IU) are an increase bone resorption markers with no effect on bone formation markers (Viljakainen et al., 2006). Protein intervention studies on markers of bone turnover indicate mixed results, with a trend towards a reduction in bone resorption markers with high protein consumption (Darling, Millward, Torgerson, Hewitt, & Lanham-New, 2009).

Malnutrition can impact approximately half of the children around the world, often occurring in underprivileged societies (Szulc et al., 2000). The lack of nutrients often results in a reduction of bone turnover, which can easily be corrected upon weight gain (Branca, Robins, Ferro-Luzzi, & Golden, 1992). In extreme cases, hyperparathyroidism is brought on as a secondary characteristic and results in high biochemical bone markers, where elevated ICTP reflects the increased breakdown of the

bone matrix and elevated levels of total AP and PICP reflect the increase in bone formation (Szulc et al., 2000).

2.4.2.d Exercise

It has been well established that PA plays a vital role in the development and maintenance of skeletal mass. Adequate amounts of PA have been shown to increase peak bone mass in youth and decrease bone loss in the elderly (Forwood & Burr, 1993). While there is a clear correlation between PA and increased BMD, the particular mechanism for the increase in BMD remains uncertain. The positive effects can vary throughout life's different stages; where the most evident benefits can be seen in pre-pubertal and pubertal skeletal growth and development (K. B. Gunter et al., 2012). Before puberty, gains in bone mass due to exercise can range between 1-6% and are considerably smaller post-puberty, thereby illustrating another beneficial aspect of incorporating PA into children's daily life (Hind & Burrows, 2007).

According to the Mechanostat Theory (Frost, 1987), bone will only respond to exercise within certain ranges of loading. This must be above or below threshold levels in order to have the adaptive response. If an adequate amount of overload is applied the load will stimulate a modeling response, in which bone is added and a new level of mechanical loading is met. This theory is based on the principle of mechanical stimuli dependence (Bailey et al., 1996).

Therefore, it is important to note that not only is the timing of PA important but also the type of activity or intensity of loading (K. B. Gunter et al., 2012). Exercise can range from leisure activities to intense and vigorous physical activities. The osteogenic

potential of exercise is dependent on the magnitude of the applied load, the rate at which the load is applied, the duration and frequency of the loading bout (J. J. Bauer & Snow, 2003).

2.5 Exercise and Bone

2.5.1 Exercise and BMD

The effects of weight bearing exercise on bone mineral acquisition in children has been studied extensively. Bonjour et al. (2009) estimated that for a 1 SD increase in population peak bone mass, fracture risk could be reduced by approximately 50%, demonstrating the importance of attaining a relatively high peak bone mass (Bonjour, Chevalley, Ferrari, & Rizzoli, 2009). Large forces applied at a very rapid rate, also known as weight bearing and high impact; appear to express the greatest benefits on bone structure and mineralization in children. Several impact exercise intervention studies during pre-puberty and puberty have elucidated this concept nicely. Gunter et al. (2008) and Fuchs et al. (2001) both showed gains in bone mass of 3.5%-8.5% following a high-impact jumping intervention; revealing some of the highest gains observed over a school year (Fuchs et al., 2001; K. Gunter, Baxter-Jones, Mirwald, Almstedt, Fuchs et al., 2008; K. Gunter, Baxter-Jones, Mirwald, Almstedt, Fuller et al., 2008). MacKelvie et al. (2001) also reported significant benefits in BMC accrual, although not as high, following a school-based impact program. An additive effect was apparent from repeated exercise exposure. BMC gains at the femoral neck and lumbar spine in early pubertal females increased from 1.5% and 3.1% to 3.7% and 4.6% following the two year jumping protocol (Mackelvie et al., 2001; MacKelvie et al., 2002). In pre-pubertal males there was

an increase in the femoral neck and total body BMC, resulting in a 4.3% difference in BMC accrual comparison to the control group following the two year jumping protocol (MacKelvie et al., 2003; MacKelvie et al., 2004). Petit et al. (2002) examined changes in bone structural properties in pre- and early- pubertal girls randomized to a jumping group in comparison to a control group. Following the 7 month program the early-pubertal jumping group showed increases in bone cross-sectional area and increased cortical thickness at the femoral neck in comparison to the control group. These structural changes led to a greater increase in the bending strength (Petit et al., 2002).

In order for older adolescents to benefit from weight-bearing, high impact exercises, a sufficiently high load and time period per session is required. Resistance training, plyometric training and step aerobics have been shown to be inadequate loads on the skeleton as there were no significant improvements in bone mass in late- and post-pubertal female adolescents (Blimkie et al., 1996; Heinonen et al., 2000; Witzke & Snow, 2000). However, improvements in the femoral neck, lumbar spine and total body BMC were evident following an 8 month jumping protocol involving approximately 300 jumps per session (Weeks et al., 2008). These results suggest that jumping protocols may provide a unique and beneficial stimulus for skeletal tissue throughout the growing years, if the load is sufficiently high. Resistance training or step aerobics may not have had a sufficiently high load that was maintained for a sufficient period of time in the adolescent age group (K.B. Gunter, Almatedt & Janz, 2012).

Although exercise is known to be a major determinant of skeletal mass, the mechanism by which the changes in bone metabolism occur is not well understood. The use of various markers of bone turnover allow for an estimation of the bone metabolic

processes and any changes in bone turnover. Inconsistencies in bone turnover may be found if the effects of short and long periods of exercise are compared, if different intensities are being compared or if preanalytical variability, uncontrollable and controllable factors, is not taken into account.

2.5.2 Exercise and Bone Turnover

2.5.2.a Long-term Exercise Training or Competitions (≥6 months) and Bone Turnover in Adults

The effect of regular exercise training or competitions on markers of bone turnover is not well studied (Table 4). Marques et al. (2011) is one of the very few studies that examined the effects of a long-term training protocol on the ratio of OPG and RANKL. Following 8 months of either resistance exercise or aerobic exercise no changes were demonstrated for either cytokine in older women (Marques et al., 2011). Vainionpaa et al. (2009) examined the effects of an impact exercise training protocol, 3 times a week, over a period of 12 months in middle-aged women. The results indicated that the impact exercise did not influence either markers of bone formation or bone resorption (Vainionpaa et al., 2009). However, Jurimae et al. (2006) and Shibata et al. (2003) both found increases in specific markers of bone formation (Jurimae, Purge, Jurimae, & von Duvillard, 2006; Shibata, Ohsawa, Watanabe, Miura, & Sato, 2003). Jurimae et al. (2006) investigated the effects of 6 months of heavy training on OC in nationally and internationally ranked male rowers. It was found that the levels of OC increased over the 6 month period of training (Jurimae et al., 2006). Shibata et al. (2006) found there to be no changes in OC or NTx following 12 months of walking or walking +

jumping protocol. There was an increase in BAP for both groups but was much more pronounced in the walking + jumping group (Shibata et al., 2003). From this limited number of studies, a pattern of the effect of long-term training on bone markers is unclear. The apparent inconsistencies may be due to the fact that different populations were studied, following different modes and intensities of exercise training.

2.5.2.b Short-term Exercise Training (≤6 months) and Bone Turnover in Adults

The effects of short-term training on bone metabolism markers have been investigated in several studies (Table 4), including jumping, aerobic and anaerobic type of exercise (Banfi et al., 2010). Erickson et al. (2010) examined the changes in bone markers in young, resistance trained males during a jumping protocol of either once a day, 3 times a week for 8 weeks (J1) or twice a day, 3 times a week for 8 weeks (J2), both resulting in the same total number of jumps. The results indicated a trend towards the J2 group to have higher levels of bone formation markers and lower levels of bone resorption markers in comparison to the J1 group, suggesting that a recovery period may restore mechanosensitivity to allow for an osteogenic effect (Erickson & Vukovich, 2010). Woitge et al. 1998 examined, in young male adults, the effects of aerobic and anaerobic running after 8 weeks of endurance or sprint training on different markers of bone resorption and formation. For the markers of bone formation, it was found that after 4 weeks of endurance training there was a significant reduction but after 8 weeks the levels had returned to baseline levels. The markers of bone resorption showed significant decreases at the 4 week point and continued to further decrease at the 8 week mark. These observations indicate that an 8 week aerobic training program may be associated with an overall suppression of bone turnover. Sprint training showed no differences in

either bone formation or resorption markers after 4 weeks of training (Woitge et al., 1998). However, by the end of the 8 weeks there were significant increases in both markers of bone formation and resorption, suggesting that there was a considerable acceleration of bone metabolism. The authors concluded that the two types of training affect bone metabolism; aerobic training exhibited decreased bone resorption thereby resulting in a net increase in bone formation, whereas anaerobic training exhibited an overall increase in bone turnover with a less marked increase in bone formation (Woitge et al., 1998). From the studies summarized in Table 4 it can be concluded that short-term training has beneficial effects on markers of bone turnover. Bone formation markers tend to increase and bone resorption markers either remain unchanged or decrease.

2.5.2.c Short-term Exercise Training (≤6 months) and Bone Turnover in Adolescents

With respect to children and adolescents Eliakim et al. (1996, 1997) examined the effects of endurance-type training in both adolescent males and females. In adolescent males, the 5-weeks of training resulted in an increase in bone formation markers and a less pronounced decrease in bone resorption markers. In adolescent females the only marker of bone turnover measured was OC and after the 5-weeks of endurance-type training there was a significant increase. From both of these studies the most important result is that a moderate intensity 5-week training protocol led to increases in bone formation markers (Eliakim et al., 1996; Eliakim, Raisz, Brasel, & Cooper, 1997).

2.5.2.d Acute Exercise and Bone Turnover

The acute effects of exercise are not as well understood (Table 5). There is inconsistency in the effects of resistance exercise, aerobic exercise and plyometric

exercise on changes in bone biomarkers. There are a limited number of studies examining the acute effects of resistance training studies on bone turnover markers. There is an apparent trend for bone resorption markers to decrease and for bone formation markers to remain unchanged (Ashizawa et al., 1998; R. S. Rogers, Dawson, Wang, Thyfault, & Hinton, 2011; Whipple et al., 2004). Endurance running, ranging from 30 minutes to full marathons, typically resulted in increased markers of bone formation and bone resorption (Brahm, Piehl-Aulin, & Ljunghall, 1997; Langberg, Skovgaard, Asp, & Kjaer, 2000; Lippi et al., 2008; Malm, Ronni-Sivula, Viinikka, & Ylikorkala, 1993; Nishiyama, Tomoeda, Ohta, Higuchi, & Matsuda, 1988; Scott et al., 2010; Scott et al., 2011; Scott et al., 2013; Thorsen, Kristoffersson, Hultdin, & Lorentzon, 1997; Welsh et al., 1997; Ziegler et al., 2005). Non-weight bearing exercise, such as cycling, does not result in a clear pattern, with some studies demonstrating increases in bone formation markers alongside increases in bone resorption markers, and others demonstrating no changes at all (Guillemant, Accarie, Peres, & Guillemant, 2004; Herrmann et al., 2007; Kristoffersson, Hultdin, Holmlund, Thorsen, & Lorentzon, 1995; Pomerants et al., 2008; Rong et al., 1997; Rudberg, Magnusson, Larsson, & Joborn, 2000; Wallace et al., 2000). Two studies examined the effects of a single session of plyometric exercise on markers of bone turnover, with no clear pattern: Lin et al. (2012) observed an increase in bone formation marker (OC) and no change in bone resorption marker (TRAP), while Rogers et al. (2011) observed no change in bone formation markers (BAP and OC) and an increase in bone resorption markers (TRAP5b and CTx) (Lin et al., 2012; R. S. Rogers et al., 2011).

The results of the studies summarized in Table 5 are strongly dependent on the type, intensity and duration of the exercise protocol. It is known that exercise stimulates osteoblast and osteoclast functioning but immediate and delayed responses need to be distinguished, suggesting that multiple samples are required throughout the recovery period (Banfi et al., 2010).

Thus far, only one study examined the effects of an acute bout of exercise on the ratio between OPG and RANKL. Ziegler et al. (2005) measured the serum levels of OPG and RANKL in middle-aged males and females following either a 15km run or a 42km run and it was found that OPG increased in the runners of the longer distance and RANKL decreased in both groups but to a further extent in the longer distance (Ziegler et al., 2005).

In several studies an acute bout of exercise was not sufficient to alter bone metabolism (Banfi et al., 2010). However, when alterations were noted, the tendency was for markers of bone formation to decrease and markers of bone resorption to increase (Banfi et al., 2010). The changes may depend on the type and duration of exercise or even the fitness status or age of participants. Because the timing of sampling is quite variable it is difficult to determine the immediate or delayed effects of acute exercise on markers of bone turnover.

The majority of the studies have included adult participants of various ages, fitness levels and both sexes. However, there is very little research examining the effects of an acute bout of exercise on bone turnover markers in children. To date there is one study by Pomerants et al. (2008) who examined the impact of acute cycling on serum

markers of bone turnover in boys at different pubertal stages. Unfortunately, this exercise mode does not elicit high external mechanical loading on the skeleton, as it is not weight bearing. Additionally, only a small number of bone markers were measured. Nevertheless, the results revealed that there were no changes in PINP or ICTP following the continuous cycle ergometer protocol at ~95% of their ventilatory threshold for 60 minutes. The highest values were seen in the early-pubertal and mid-pubertal group of boys (Pomerants et al., 2008).

Table 3. Effects of long term training or competition (≥ 6 months) on markers of bone formation and bone resorption

Reference	Bone Marker	F/R	Medium	Sample	Exercise type and/or intensity	Sample Timing(s)	Comments:
Jurimae et al., 2006	OC	F	Plasma	*n* = 12 elite male rowers 20.8±3.0 years	- 6 months of high-volume, low-intensity strength training	Baseline Post Training – 6 months	- OC ↑ after 6 months
Marques et al., 2011	OPG	F	Serum	*n* = 71 sedentary older women 67.3±5.2 years	- 3x/week, 60 min, 32 weeks - Resistance (RE) – full body, 2 sets, 10-15 reps, 50-80% 1RM - Aerobic (AE) – dynamic activities (stepping, skipping, graded walking, jogging, dancing, aerobics)	Baseline Post Training 7:30am – 9:30am	- OPG and RANKL no change after 8 months
	RANKL	R					
Shibata et al., 2003	OC	F	Plasma	*n* = 43 women 37±7 years	- Walking group (WG) – 10 000 steps/day - Jumping group (WJG) – 10 000 steps + 10 jumps/day - 12 months	Baseline Post training, 12 months	- OC no change - BAP ↑ in both groups but was higher in the WJG group - NTx no change
	BAP						
	NTx	R					
Vainionpaa et al., 2009	PINP	F	Serum	RA women 35-40 years *n* = 37 exercise group *n* = 39 control group	- 60 min. impact ex., 3x/week, 12 months - Step patterns, stamping, jumping, running and walking	Baseline 6 months 12 months 8:00am – 11:00am	- PINP and TRAP5b no change
	TRAP5b	R					

F – formation, R – resorption, RA – recreationally active

(Jurimae et al., 2006; Marques et al., 2011; Shibata et al., 2003; Vainionpaa et al., 2009)

Table 4. Effects of short term training or competition (≤ 6 months) on markers of bone formation and bone resorption

Reference	Bone Marker	F/R	Medium	Sample	Exercise type and/or intensity	Sample Timing(s)	Comments:
Eliakim et al., 1997	OC	F	Serum	*n* = 44 late pubertal males 15-17 years	5 weeks endurance type training (running, aerobic dance, competitive sports, occasional weights)	Baseline – 1 week before (early morning) Post Training – During the week after completion (early morning)	- OC, BAP and PICP ↑ significantly - Dpd or CTX no change - NTX ↓ significantly
	BAP						
	PICP						
	Dpd	R	Urine				
	CTX						
	NTX						
Eliakim et al., 1996	OC	F	Serum	*n* = 44 late pubertal females 15-17 years	5 weeks endurance type training (running, aerobic dance, competitive sports, occasional weights)	Baseline Post Training	- OC ↑ significantly
Erickson & Vukovich, 2010	BAP	F	Serum	*n* = 21 resistance trained males 24.1±2.5 years	- Group 1 – jumping 1/day, 3x/week - Group 2 – jumping 2/day, 3x/week	Baseline 4 weeks training 8 weeks training	- BAP ↑ significantly in both groups after 8 weeks - CTX ↓ significantly in both groups after 8 weeks - Trend for J2 to have ↑ formation and ↓ resorption
	CTX	R					
Morgan et al., 2011	OC	F	Serum	*n* = 29 female athletes 19.7±0.82 *n* = 4 sedentary females 19.3±1.89	- High Impact - Medium Impact - Non-impact	Baseline – 8:00am-10:00am Mid-Season Post-Season (within 2 weeks of completion)	- OC and BAP ↑ in the high and medium group compared to control but no changes over time - NTX no difference between groups or change over time
	BAP						
	NTX	R					
Woitge et al., 1998	OC	F	Serum	*n* = 22 sedentary males 20-30 years	- Group 1 – endurance running 40-60min , HR at 60-85% VO_2max - Group 2 – sprints at 90-100% speed - Group 3 – control	Baseline 4 weeks training 8 weeks training	- Group 1 – OC and BAP ↓ at 4 weeks, returned to baseline by 8 weeks, Pyr and Dpd ↓ at 4 weeks and ↓↓ at 8 weeks - Group 2 – OC, BAP and Pyr ↑ after 8 weeks
	BAP						
	Pyr	R	Urine				
	Dpd						

- children, F – formation, R – resorption, HR – Heart rate

(Eliakim et al., 1996; Eliakim et al., 1996; Eliakim et al., 1997; Erickson & Vukovich, 2010; Morgan & Weiss Jarrett, 2011; Morgan & Weiss Jarrett, 2011; Woitge et al., 1998; Woitge et al., 1998)

Table 5. The acute effects of physical activity on markers of bone formation and bone resorption

Reference	Bone Marker	F/R	Medium	Sample	Exercise type and/or intensity	Sample Timing(s)	Comments:
Ashizawa et al., 1998	OC BAP PICP TRAP	F R 	Serum	*n* = 14 UT males 25±0.7 years	RE (3 sets of 10 reps – 60% of RM for 1st set and 80% of RM for 2nd and 3rd sets) Onset of exercise 4:00pm	Baseline – 8:00am Post ex. – 5 min., 1, 2 and 3 days	- OC no change - ↓ BAP 2 and 3 days post ex. - PICP and BAP ↓ ex. day and ↓↓ 1 day post ex. – baseline by recovery
Brahm et al., 1996	OC BAP PICP ICTP	F R 	Serum	*n* = 10 active males 22-52 years *n* = 10 active females 22-55 years	Long distance running – mean distance of 28km Onset of exercise unknown	Baseline – morning 24hrs pre ex. Post ex. – morning 1 and 2 days	- ↑ OC 1 day post ex. in males only - BAP no change - ↓ PICP post run but returned to baseline 2 days post ex. in females only
Banfi et al., 2012	OPG RANKL	F R	Serum	*n* = 30 professional male rugby players 26±2 years	Training camp – field practice	Baseline Post ex.	- OPG and RANKL showed non-significant ↑ post ex.
Guillemant et al., 2004	BAP CTX	F R	Serum	*n* = 12 T male triathletes 23-37 years	1 hour cycle ergometer at 80% of VO_2max Onset of exercise 9:30am	Baseline – 8:30am, 9:30am During ex. – 10:00am Post ex. – immediately, 30 min., 1 hour and 2 hours	- BAP no change - ↑ CTX 30min post ex. through to 2 hours post ex. - Ingestion of high-calcium mineral water suppressed CTX response

F – formation, R – resorption, RE – resistance exercise, RM – repetition maximum, UT – untrained, T – trained, PL – plyometrics, IR – intermittent running, CON – control, RA – recreationally active, WBV – whole body vibrations

Table 5. The acute effects of physical activity on markers of bone formation and bone resorption continued.

Reference	Bone Marker	F/R	Medium	Sample	Exercise type and/or intensity	Sample Timing(s)	Comments:
Herrmann et al., 2007	OC	F	Serum	*n* = 32 (MA, male athletes 26±4 years; MCo, male controls 36±21 years; FA, female athletes 24±3 years; FCo, female controls 25±2 years)	Incremental anaerobic threshold cycle ergometer – started at 50W, increase of 5-W (males) or 30W (females) every 3 minutes	Baseline Post ex. – 3 hours and 24 hours	- Exercise at 75% intensity↓ OC and PINP in all groups post ex. - CTX and TRAP not consistently modified
	PINP						
	CTX	R					
	TRAP						
Kristofferson et al., 1995	OC	F	Serum	*n* = 7 T males 22 years	Modified Wingate test – cycle ergometer Onset 9:00am-1:00pm	Baseline – 1 hour pre ex. Post ex. – 5 and 60 mins.	- No changes in any of the markers
	PICP						
	ICTP	R					
Langberg et al., 2000	PICP	F	Plasma	*n* = 17 male runners 23-48 years	Marathon running (onset of 9:30am)	Baseline – 7:00am (1 week prior) Post ex. – immediately, 1, 2, 3, 4, 5, 6 days (7:00am)	- PICP ↓ immediately post ex., ↑ and peaked day 3, returned to baseline day 5 - ICTP short ↑ immediately post ex. but returned to baseline by 24 hours
	ICTP	R					
Lippi et al., 2008	OC	F	Serum	*n* = 15 T males 47 years	Half marathon (21km) under competitive conditions	Baseline Post ex. – immediately, 2, 6 and 24 hours	- ↑ OC immediately post ex. but returned to baseline by 3 hours
Lin et al., 2012	OC	F	Serum	*n* = 24 UT males 25±0.7 years (PL group, IR group and CON group)	Plyometric jumping – 5 sets forward jumping and lateral jumping	Baseline – 8:00am Post ex. – 5min, 15min, 1, 3, 6, 24, 48 and 72 hours	- ↑ OC vs. CON at 5min and 1 hour post ex. - No significant differences in TRAP vs. CON
	TRAP	R			Interval running – 200m x 10 (onset of 9:00am)		- No significant differences in OC or TRAP vs. CON

F- formation, R – resorption, RE – resistance exercise, RM – repetition maximum, UT – untrained, T – trained, PL – plyometrics, IR – intermittent running, CON – control, RA – recreationally active, WBV – whole body vibrations

Table 5. The acute effects of physical activity on markers of bone formation and bone resorption continued.

Reference	Bone Marker	F/R	Medium	Sample	Exercise type and/or intensity	Sample Timing(s)	Comments:
Malm et al., 1993	OC	F	Serum	*n* = 8 male marathon runners 25-41 years *n* = 15 female marathon runners 23-55 years	Marathon run	Baseline Post ex. – immediately, 1, 3 and 5 days (between 3:00pm and 7:00pm)	- OC ↓ 1 day post ex. and continued to day 3 in males and remained lower in females - BAP ↓ in females only immediately post ex. and remained lower - Ca ↓ immediately post ex. but returned to baseline by day 1 in males and females
	BAP						
	Calcium	R	Urine				
	Hyp						
Nishiyama et al., 1988	OC	F	Serum	*n* = 18 (A, athletic male volleyball players; NA, non-athletic sedentary males) 20-24 years	30 min running ergometer at approximately 50% of maximum capacity (onset 5:00pm)	Baseline – 5:00pm Post ex. – immediately and 1 hour	- A ↑ baseline values vs. NA - A OC ↑ 1 hour post ex. - NA OC ↑ immediately post ex. and returned to baseline values by 1 hour
Pomerants et al., 2008	PINP	F	Serum	*n* = 60 boys 10-18 years	Acute cycle ergometer (30 min ~95% of ventilator threshold) (onset between 3:00pm and 5:00pm)	Baseline – 3:00pm-5:00pm Post ex. – immediately and 30 min.	- No change in either PINP or ICTP at any time point - Pubertal group II (Tanner 2 and 3) significantly higher PINP and ICTP concentrations vs. pre-pubertal (Tanner 1) and pubertal group III (Tanner 4 and 5)
	ICTP	R					

F – formation, R – resorption, RE – resistance exercise, RM – repetition maximum, UT – untrained, T – trained, PL – plyometrics, IR – intermittent running, CON – control, RA – recreationally active, WBV – whole body vibrations, A – athletic, NA – non-athletic, - children

Table 5. The acute effects of physical activity on markers of bone formation and bone resorption continued.

Reference	Bone Marker	F/R	Medium	Sample	Exercise type and/or intensity	Sample Timing(s)	Comments:
Rogers et al., 2011	BAP	F	Plasma	*n* = 12 UT males 24-62 years	Plyometric jumping and bounding – 1 set, 10 reps or 2 sets, 5 reps Resistance – 3 sets, 10 reps (1st set 60% RM, 2nd and 3rd set 80% RM) (Onset between 6:00am-7:00am)	Baseline – 6:00am-7:00am Post ex. – immediately, 15 min., 30min., 1 and 2 hours	- BAP unchanged but the natural morning ↓ was prevented - No change in OC - TRAP5b ↓ for both PLY and RT at 15 min. and 30 min. post ex. but returned to baseline values 1 hour post ex. - CTX ↑ at 1 hour, ↓ 2 hour, ↑ 24 hour post ex.
	OC						
	TRAP5b	R					
	CTX						
Rong et al., 1997	OC	F	Serum	*n* = 8 UT males 23±3 years	Cycle ergometer – E55% or E85% VO_2max 45 or 15min – STR (supine leg press)	Baseline – 7:00am-8:00am (fasted) Last minute of exercise Post ex. – 1, 4 and 24 hours	- ICTP ↓ 4 hours post ex. after all types of exercise - OC ↑ 4 hours post ex. after E55%
	ICTP	R					
Rudberg et al., 2000	OC	F	Serum	*n* = 15 (CY, cycle ergometer group; JG, jogging group) RA postmenopausal women 57±4 years	Cycle ergometer until exhaustion – increase of 30W every 6min 30-40min of jogging – 4-6km (onset – afternoon)	Baseline – afternoon Post ex. – immediately and 20 min.	- OC no change - BAP (isoform B2) ↑ immediately post ex. in both groups - ICTP no change
	BAP						
	ICTP	R					

F – formation, R – resorption, RE – resistance exercise, RM – repetition maximum, UT – untrained, T – trained, PL – plyometrics, IR – intermittent running, CON – control, RA – recreationally active, WBV – whole body vibrations

Table 5. The acute effects of physical activity on markers of bone formation and bone resorption continued.

Reference	Bone Marker	F/R	Medium	Sample	Exercise type and/or intensity	Sample Timing(s)	Comments:
Scott et al., 2010	OPG	F	Plasma	*n* = 21 RA males (RA, exercise group 30±3 years; CON, control group 26±3) *n* = 10 ET males 31±3 years	Intermittent exhaustive running (onset ~8:00am)	- Baseline – 8:00am - During ex. – 20, 40 and 60min. - Post ex. – Immediately, 30min., 1, 1.5 and 2 hours - 1-4 days post ex. – fasting ~8:00am/day	- OPG ↑ during exercise, immediately and 2 hours post ex. in both groups - BAP and PINP no change - β-CTX ↑ 1-4 days post ex. in both groups vs. control
	BAP		Serum				
	PINP						
	β-CTX	R					
Scott et al., 2011	BAP	F	Serum	*n* = 10 RA males 28±4 years	60min treadmill running at 55%, 65% and 75% of VO_2max (onset 8:15am)	- Baseline – 8:00am - During ex. – 20, 40 and 60min. - Post ex. – 30min., 1, 2 and 3 hours - 1-4 days post ex. – fasting 8:00am/day	- BAP not affected by exercise intensity but ↑ at day 3 and 4 post ex. - PINP ↑ during ex. for all intensities but ↓ quickly in recovery to baseline - OC not affected by exercise intensity but ↓ 3 days post ex. - OPG not affected by exercise intensity but ↑ 20 min. during ex. and remained ↑ 3 hours post ex. - β-CTX not ↑ by ex. but was higher in 55% and 65% ex.
	PINP		Plasma				
	OC						
	OPG						
	β-CTX	R					

RE – resistance exercise, RM – repetition maximum, UT – untrained, T – trained, PL – plyometrics, IR – intermittent running, CON – control, RA – recreationally active, WBV – whole body vibrations

Table 5. The acute effects of physical activity on markers of bone formation and bone resorption continued.

Reference	Bone Marker	F/R	Medium	Sample	Exercise type and/or intensity	Sample Timing(s)	Comments:
Scott et al., 2013	β-CTX	R	Serum	*n* = 10 RA males 26±5 years	Two 60 min. bouts of treadmill running at 65% VO_2 max separated by a recovery of either 23 hours (LONG) or 3 hours (SHORT) LONG – onset 2:30pm SHORT – onset 10:30am	LONG Ex. Day 1: - Baseline – 8:00am - Pre ex. – 2:15pm - Post ex. – Immediately, 1, 2 and 3 hours LONG Ex. Day 2: - Baseline – 8:00am - Pre ex. – 2:15pm - Post ex. – Immediately, 1, 2 and 3 hours SHORT Ex. Day 1: - Baseline – 8:00am - Pre ex. – 10:15am - Post ex. – Immediately, 1, 2 and 3 hours LONG & SHORT - 1-4 days post ex. – 8:00am	- β-CTX, PINP and BAP no change - OPG ↑ with all exercise bouts, second bout not altered by recovery duration
	PINP	F					
	OPG						
	BAP		Plasma				
Sherk et al., 2013	BAP	F	Serum	*n* = 10 RA females 20.7±0.2 years	RE-only – 3 sets of 10 repetitions at 80% 1RM (4 lower body, 2 upper body) WBV + RE – same protocol and 5 one min. intervals at 20 Hz Onset 7:00am	RE only: - Baseline – 7:00am - Post-ex. – immediately, 30 min. WBV + RE: - Baseline – 7:00am - Post-ex. – immediately WBV, immediately RE and 30 min. RE	- BAP no change both groups - TRAP5b ↑ post WBV but ↓ immediately post to 30 min. RE in both groups - CTX ↓ post WBV and post 30 min RE in WBV+RE group
	TRAP5b	R					
	CTX						

RE – resistance exercise, RM – repetition maximum, UT – untrained, T – trained, PL – plyometrics, IR – intermittent running, CON – control, RA – recreationally active, WBV – whole body vibrations

Table 5. The acute effects of physical activity on markers of bone formation and bone resorption continued.

Reference	Bone Marker	F/R	Medium	Sample	Exercise type and/or intensity	Sample Timing(s)	Comments:
Thorsen et al., 1997	OC	F	Serum	*n* = 14 sedentary females 25.2±0.6 years	Outdoor jogging at 50% VO_2max, estimated by 50% heart rate max Onset 8:30am-9:30am	- Baseline – 15 min. pre ex. - Post ex. – 1, 24 and 72 hours	- OC ↑ 1 hour post ex. but returned to baseline 24 hours post ex. - PICP ↓ 1 hour post ex. but ↑ 24, 72 hours post ex. - ICTP ↑ 24, 72 hours post ex.
	PICP						
	ICTP	R					
Tosun et al., 2006	OC	F	Plasma	*n* = 9 sedentary females 28±2.2 years	- Walking (W) – 30 min. submax treadmill walking - Walking with 5kg (WE) – 30 min. submax treadmill walking Onset between 9:00am-11:00am	- Baseline – 9:00am-11:00am - Post ex. – immediately, 45 min (OC, PINP, PICP, ICTP), 1 hour (Dpd), 24 hours (BAP, Dpd)	- OC, PICP, PINP and ICTP no changes - BAP ↓ 24 hours post ex. in W group and ↑ 24 hours post ex. in WE group
	PICP						
	PINP						
	BAP	R					
	ICTP						
	Dpd		Urine				
Wallace et al., 2000	OC	F	Serum	*n* = 17 RA males 26.9±1.5 years	Cycle ergometer incremental protocol Onset late afternoon or evening	- Baseline – 3hr fasting afternoon-evening 30min. and immediately prior to ex. - During ex. – 15 and 30 min. - Post ex. – 45, 60, 75, 90 and 120 min.	- OC no change - BAP, PICP and ICTP ↑ in response to ex.
	BAP						
	PICP						
	ICTP	R					

RE – resistance exercise, RM – repetition maximum, UT – untrained, T – trained, PL – plyometrics, IR – intermittent running, CON – control, RA – recreationally active, WBV – whole body vibrations

Table 5. The acute effects of physical activity on markers of bone formation and bone resorption continued.

Reference	Bone Marker	F/R	Medium	Sample	Exercise type and/or intensity	Sample Timing(s)	Comments:
Welsh et al., 1997	OC	F	Serum	*n* = 10 sedentary males 25.7±4.5 years	30 min. on motor driven treadmill 60% of predicted max heart rate Onset between 8:00am-8:30am	Serum: - Baseline – 8:00am-8:30am - Post ex. – 30 min., 1, 8, 24 and 32 hours Urine: - 1 day pre ex., ex. day and 1 day post ex.	- OC and BAP no change - Pyr and Dpd ↑ day of ex. and ↑↑ 1 day post ex.
	BAP						
	Pyr	R	Urine				
	Dpd						
Whipple et al., 2004	BAP	F	Serum	*n* = 9 RA males 21.9±1.2 years	3 sets of 10 – 50, 75 and 100% of 10 RM (bench press, leg press, lateral pull down, seated row, leg curl, back extension and arm curl) Onset is unknown	Serum: - Baseline - Post ex. – immediately, 1, 8, 24 and 48 hours Urine: - 1 day pre ex., ex. day, 1 day post ex. and 2 days post ex.	- BAP and PICP no change - sNTx ↓ 1 and 8 hours post ex. - uNTx no change - BAP : sNTx ↑ at 1 and 8 hours post ex. - Returned to baseline 24 hours post ex.
	PICP						
	sNTx	R					
	uNTx		Urine				
Ziegler et al., 2005	OPG	F	Plasma	*n* = 31 recreational male (43.7±10.9 years) and female (37.3±8.6 years) runners	Marathon or half marathon run Onset is unknown	- Baseline – 30min. prior to run - Post ex. – 30 min. within finishing run	- OPG ↑ in full marathon runners - RANKL ↓ in half and full marathon runners
	RANKL	R					

RE – resistance exercise, RM – repetition maximum, UT – untrained, T – trained, PL – plyometrics, IR – intermittent running, CON – control, RA – recreationally active, WBV – whole body vibrations, sNTx – serum NTx, uNTx – urinary NTx

(Ashizawa et al., 1998; Banfi, Corsi, & Galliera, 2012; Brahm, Piehl-Aulin, & Ljunghall, 1996; Guillemant et al., 2004; Herrmann et al., 2007; Kristoffersson et al., 1995; Langberg et al., 2000; Lin et al., 2012; Malm et al., 1993; Nishiyama et al., 1988; Pomerants et al., 2008; R. S. Rogers et al., 2011; Rong et al., 1997; Rudberg et al., 2000; Scott et al., 2010; Scott et al., 2011; Scott et al., 2013; Sherk et al., 2013; Thorsen et al., 1997; Tosun, Bolukbasi, Cingi, Beyazova, & Unlu, 2006; Welsh et al., 1997; Whipple et al., 2004; Ziegler et al., 2005)

Chapter 3: Methods

3.1 Participants

This study and all related procedures received ethical clearance from the Brock University Research Ethics Board. Participants were recruited through poster advertisements, flyers and information packages to local elementary schools and universities/colleges throughout the Niagara region. Recruitment of the participants focused on recreationally active young men and pre-pubescent boys to limit the impact of pubertal and post peak bone mass fluctuations in bone markers and bone strength measures.

After showing interest in the study, participants, and their parents for children, were provided with a thorough description of the purpose, methods and any potential risks of the study. Exclusion criteria included factors that can influence properties of bone (1) BMI $\geq 85^{th}$ percentile for their age, (2) a body fat percentage $\geq 30\%$, (3) previous or current fracture, (4) premature growth or growth delay and (5) use of pharmaceuticals (Antiresorptives, bisphosphonates, corticosteroids, anticonvulsants and GnRH agonists). A total of 30 participants completed the study, 12 of which were boys (10.2 ± 0.4 years) and 18 young men (22.5 ± 0.7 years).

3.2 Procedures

Participants were instructed to refrain from alcohol and caffeine for a minimum of 6 hours prior and vigorous or impact exercise for a minimum of 24 hours prior to testing. Participants were invited to the Applied Physiology Exercise Physiology Laboratory for two separate visits. The first visit took place at 9:00am for all participants, in order to

control for circadian rhythm variation. Upon arrival, participants and parents were introduced to the purpose of the study, the methods involved and any potential benefits or discomforts and an informed consent/assent was then signed by either the participant or parent/guardian. Afterwards, the resting baseline blood sample was drawn, using a standard venipuncture technique and a questionnaire package was completed, including medical information, maturation, leisure-time physical activity, past-year physical activity and dietary intake. Children participants were assisted with the completion of the questionnaires by either the investigator or parent/guardian as necessary. The plyometric exercise protocol was then completed and followed by two additional blood samples; five minutes post-exercise and one hour post exercise. During the second visit, the last blood sample 24 hours post-exercise was taken and anthropometric measures of height, sitting height (children only), weight and body composition were taken. Subsequently, quantitative ultrasound (QUS) was used to determine bone strength of the tibia and radii.

3.3 Measurements

3.3.1 Anthropometry

All measures of height, sitting height, weight and body composition were assessed by the same investigator for all participants. Height was measured, using a stadiometer, to the nearest 0.1cm with no shoes and light clothing. For maturational indications, sitting height was measured to the nearest 0.1cm for the pre-pubescent males using the stadiometer and a table. This was measured by subtracting the height of the table from the combined height of the table and sitting participant. Body mass (kg) was measured using the InBody520 bioelectrical impedance analysis (BIA) system

(Biospace.2008.). Body mass index (BMI) was calculated by dividing the participants' mass (kg) by their height squared (m^2). Percent body fat (PBF) and lean body mass (LBM) were assessed using skinfold thickness measurements. PBF was estimated using the Slaughter et al. (1988) equations, based on skinfold thickness at two sites, triceps and subscapular, using a Harpenden caliper. All skinfold measurements were made on the right side of the body. Each site was measured in triplicate and the median was recorded. PBF was then calculated using Slaughter's standardized equations, which are based on sex and maturity level (Slaughter et al., 1988).

3.3.2 Indicators of Maturity

Sexual maturity was determined using secondary sexual characteristics, as defined by Tanner (1962) (self-assessed using drawings) (Tanner, 1962). Refer to appendix. Pubic hair development was used to determine sexual maturity. Skeletal maturity was determined by calculating the years from the age of peak height velocity (PHV), using height, sitting height, leg length and body mass as suggested by Mirwald et al. 2002 (Mirwald, Baxter-Jones, Bailey, & Beunen, 2002).

3.3.3 Habitual Physical Activity and Dietary Intake Measures

Habitual physical activity was assessed using three different questionnaires. The Godin-Shephard Leisure Time Exercise Questionnaire was designed to evaluate leisure time physical activity using intensity indicators such as light, moderate or strenuous (Godin & Shephard, 1985). The questionnaire was administered to the participants to assess the weekly physical activity metabolic equivalent (WA_{eq}) by indicating the number of times they engaged in at least 15 minutes of each intensity level. The number of each

intensity level was then multiplied by known energy consumption values to determine the metabolic equivalents (Godin & Shephard, 1985). This method of physical activity assessment has shown to be a valid and reliable tool in children and adolescents as well as in adult male and female populations (Sallis, Buono, Roby, Micale, & Nelson, 1993).

The Bone-Specific Physical Activity Questionnaire (BPAQ) was administered to the participants to record type, frequency and years of current and historical physical activity involvement (Weeks & Beck, 2008). Scoring is based on an algorithm which takes into account the osteogenic index principle described by Turner and Robling and ground reaction forces (GRF) (Turner & Robling, 2003). It should be noted that this questionnaire was developed in a small sample of adults and may not predict bone parameters in other populations, such as children (Farr, Lee, Blew, Lohman, & Going, 2011).

The Past Year Physical Activity Questionnaire (PYPAQ) was administered to survey the time spent in leisure and sporting activities in the past year (Aaron et al., 1995). The participants were asked to indicate all of the leisure-time activities that they had engaged in over the past year for at least 10 times, followed by details regarding the frequency and duration of each activity listed. This physical activity assessment tool has been validated in adolescents (Aaron et al., 1995). In addition, it has also been compared to the BPAQ in girls and it was shown that the PYPAQ was a stronger predictor of indices of bone strength (Farr et al., 2011). Both questionnaires were included to ensure all measures of bone-relevant physical activity were taken into consideration.

Dietary intake was evaluated using the 24-hour Nutrition Recall Questionnaire. The participants were asked to recall a typical day of eating. With the use of a variety of visual aids, the different portion sizes were determined to ensure the most accurate representation of each participant's nutritional intake. Nutritionist Pro™ (Axxya Systems, USA) was used to analyze each participant's dietary intake, ultimately providing an estimate for total caloric intake (kcal), calcium intake (mg), vitamin D intake (μg) as well as caffeine intake (mg).

3.3.4 Quantitative Ultrasound (QUS)

QUS was performed in order to examine overall bone health. The SOS measurement is an indicator of bone strength, reflecting bone mineral density, elasticity and microarchitectural structure (Barkmann et al., 2000). The QUS method has been shown in several studies to be a sensitive diagnostic tool in bone fragility and changes in bone strength. QUS has been used to predict vertebral and hip fractures and *in vitro* studies have indicated the capability of measuring previously unquantified properties of bone fragility (D. C. Bauer, Gluer, Genant, & Stone, 1995; Gluer, Wu, & Genant, 1993; Njeh, Boivin, & Langton, 1997). It has also been shown to be a sensitive tool to detect changes in tibial bone properties among pre-pubertal boys over an 8-month period (Falk et al., 2000).

This method is a simple, inexpensive, non-invasive and radiation-free way of investigating and reflecting bone strength. Because QUS is not influenced by the size of bone it is advantageous when assessing children (Barkmann et al., 2000; Schoenau et al., 2004).

Skeletal properties were assessed by measuring bone speed of sound (SOS) using QUS (Sunlight Omnisense^{TM} 7000S, Sunlight Medical, Israel) at the distal 1/3 of the radius and the mid-shaft of the tibia, on both the dominant and non-dominant limbs. The measurement site of the radius was determined as the midpoint between the olecranon process and the tip of the third distal phalanx, while the measurement site for the tibia was determined as the midpoint between the bottom of the calcaneous and the top of the patella with the knee at a 90 degree angle. The system contains a main unit and a hand-held probe, which is used to measure the SOS (m/s) of the ultrasound waves along cortical bone. Z-scores between -1 and 1 standard deviations indicate healthy bone properties in relation to the available norms. Preceding any measurements the system underwent calibration procedures according to the manufacturer's protocol for quality control procedures. The probe is positioned to run, at a critical angle, along the bone around the arc of the radius and the tibia.

3.3.5 Exercise Protocol

Upon completion of the medical screening questionnaire, participants were asked to wear a heart rate monitor and an accelerometer. The heart rate monitor included the wrist watch and the transmission strap worn around the chest. Accelerometers were used to record accelerations (counts) in the vertical or horizontal axes. The accelerometers were programmed to record activity counts at 1 second epochs. The exercise protocol was designed to provide high impact, weight-bearing loads in the form of circuit training stations and was modified from the protocol used by MacKelvie et al, (2001) in the long-term school-based interventions (Mackelvie et al., 2001).

The participants began with a warm-up activity that consisted of a 5 minute incremental cycle ergometer protocol. The adults started at 120 watts (W) and increased by 30W every minute and the children started at 25W and increased by 15W every minute. Upon completion of the warm-up, a thorough explanation and demonstration of the exercises was given to the participants who were also able to familiarize themselves with the exercises.

The participants were then instructed to rotate through the five stations, which were comprised of, in this order, drop jumps, lunge jumps, hurdle jumps, single-leg hops and jumping jacks. The drop jumps were 75cm in height for the adults and 40cm in height for the children. The hurdle jumps were 40cm in height for the adults and 15cm in height for the children. For both adults and children, 3 sets of 8 repetitions were completed with a recovery period of at least three minutes between each set. The participants were asked to jump to their highest potential for each individual jump. The heart rates were recorded after each exercise and rest period. By following this protocol, an approximation of 144 jumps was executed upon completion (Mackelvie et al., 2001). McKay et al. (1998) demonstrated that the ground reaction forces involved in these circuit stations characteristically involved forces up to 3.5- to 5-times body weight (McKay, Bailey, Mirwald, Davison, & Faulkner, 1998).

3.3.6 Biochemical Markers of Bone Turnover

Resting venous blood samples were drawn using a standard venipuncture technique from a vein in the antecubital fossa between 9:00am and 9:30am. The morning hours were chosen for the baseline measurement to control for circadian rhythm

variations. All participants were asked to refrain from physical activity 24 hours prior to the resting blood sample. A total of 10ml of blood was drawn per sample and was collected using serum tubes (BD Vacutainer®). The blood was centrifuged at 3000rpm, 4°C for a total of fifteen minutes after waiting at least 1 hour post blood draw at room temperature. The waiting time rarely exceeded the 1 hour post draw. The serum was separated, removed from the sample tube and aliquoted into six 0.5ml polyethylene tubes. The tubes were then stored at -80°C until analysis.

Serum was analyzed for biochemical markers of bone turnover. The following bone formation markers were measured using enzyme-linked immunosorbent assay (ELISA) kits: bone-specific alkaline phosphatase (BAP) and osteoprotegerin (OPG). Serum BAP immunoassay appears to be the method of choice due to its high specificity and precision and has been shown to be a sensitive indication tool of bone formation (Gomez et al., 1995). OPG is a key regulator of osteoclastogenesis and the commercial OPG assay precision is within the acceptable ranges with the latest generation having low detection limits (Haufbauer & Heufelder, 2001; Kholsa, 2001; A. Rogers & Eastell, 2005). The following bone resorption markers were measured: N-telopeptides of type I collagen (NTx) and receptor activator of nuclear factor κB ligand (RANKL). Serum NTx has been shown to provide a responsive index of human bone resorption in healthy populations (Clemens, Herrick, Singer, & Eyre, 1997). RANKL is a pre-resorptive factor that stimulates osteoclast differentiation (Hofbauer & Heufelder, 2001; Kholsa, 2001). RANKL assay sensitivity has recently been improved with the use of a secondary antibody and a modified detection method (A. Rogers & Eastell, 2005). OPG and RANKL are not traditional measures of bone turnover but can provide insight into the

early phase of bone cellular response to exercise. At present, there are no such data related to high impact exercise in children or in adults.

All samples were thawed and ELISA kits brought to room temperature prior to the onset of the procedures. All analyses were performed in duplicate and the absorbencies were read using a microplate reader.

Bone-specific alkaline phosphatase (BAP) was assayed using four Ostase® BAP Immunoenzymetric Assays (Immunodiagnostics Systems, Fountain Hills, AZ, USA). Prior to the onset of the analysis, all reagents were thoroughly mixed by gentle agitation or swirling, the Wash Solution was prepared and specific samples were diluted. The Wash Solution was prepared by adding the Wash Concentrate to 950ml of deionized water and mixing it using a magnetic stirrer for a minimum of 5 minutes. Because childrens' values would fall above the standard curve, their samples needed to be diluted 1:3 and mixed thoroughly using the vortex (Hui et al., 2003).

Upon completion of the preparatory steps 50µl of each Calibrator, Control and sample were pipetted into each assigned well. Using a multichannel pipette, 100µl of Conjugate was then pipetted into each well. A plate sealer was applied to the microplate which was then incubated for 1 hour at room temperature using a horizontal rotator set at 500rpm. At the end of the incubation period the microplate was washed 3 times with the Wash Solution. After the final wash, the microplate was inverted and tapped strongly against paper towel. Using a multichannel pipette, 150µl of Substrate reagent was delivered to each well and without delay, was incubated for 13-15 minutes at room temperature using a horizontal rotator set at 500rpm. After the incubation period using a multichannel pipette, 100µl of Quench Reagent was added to each well. Within 1 hour of

adding the Quench Reagent, the microplate absorbance was read using a microwell plate reader at 405nm.

Assay performance can be verified if the following criteria were met: (1) recovery of the control concentrations fell within the stated ranges and (2) the coefficient of variation (%CV) of the 405nm absorbance readings for each calibrator and control sample was less than 10%.

After the absorbance values were determined, a standard curve was produced using the calibrator points. The concentration values for the controls and samples of BAP, expressed in μg/L, were then determined from the calibration curve using a third order polynomial equation, with an average r^2 value of 0.99543(0.9882-0.9999). Because the children's samples were diluted in the preparatory steps, the dilution factor needed to be taken into consideration when calculating the values. The ranges of the low and high controls set by the kit were 7.6-11.4μg/L and 39.2-58.8μg/L, which all of the assayed controls fit within. The intra-assay CVs were 3.3%, 3.2%, 3.3% and 6.9%, averaging 4.2%. The average inter-assay CV for 4 plates was 1.4%.

Osteoprotegerin (OPG) was assayed using four Human OPG ELISA kits (Biovendor Research and Diagnostics Products, Asheville, NC, USA). Prior to the assay procedure the Standards, Controls, Wash Solution Concentrate and samples were all prepared. The lyophilized Master Standard was reconstituted with Dilution Buffer and was left to dissolve for at least 15 minutes with occasional gentle shaking. The set of standards was prepared using the amount of Dilution Buffer stated in the product data sheet. Once prepared, the standards were then further diluted 3x with Dilution Buffer, and

were ready to be aliquoted. The lyophilized Quality Controls were reconstituted with deionized water and were left to dissolve for at least 15 minutes with occasional gentle shaking. The prepared Quality Controls were diluted 3x with Dilution Buffer and were then ready to be aliquoted. The Wash Solution Concentrate was prepared by diluting it 10x or ten-fold in deionized water for a 1x working solution. Samples were diluted 3x with Dilution Buffer and mixed thoroughly using the vortex.

Following the preparatory steps, 100µl of the diluted Standards, Quality Controls and samples were distributed to the bottom of each assigned well. A plate sealer was applied to the microplate, which was then incubated at room temperature for 1 hour, shaking at 300rpm on an orbital microplate shaker. At the end of the incubation period the wells were washed 3 times using the Wash Solution. After the final wash, the microplate was inverted and tapped strongly against paper towel. Using a multichannel pipette, 100µl of the Biotin Labelled Antibody was added to each well and was then incubated for another hour at room temperature, shaking at 300rpm on an orbital plate shaker. Following this incubation period, 100µl of Streptavidin-HRP Conjugate was added to each well using the multichannel pipette. The microplate was then incubated for 30 minutes, shaking at 300rpm on an orbital plate shaker. The last washing step occurred after this incubation period, where the microplate was washed 3 times using the Wash Solution and was inverted and tapped strongly against paper towel. Using the multichannel pipette 100µl of the Substrate Solution was added to each well. The microplate was then covered with a plate sealer as well as aluminum foil to avoid exposure to direct sunlight. It was incubated for 10 minutes at room temperature and was not to be shaken. During this step a blue colour should have formed and to stop the colour

development 100µl of Stop Solution was added to each well using the multichannel pipette. Within 5 minutes of adding the Stop Solution absorbance was read using a microwell reader at 450nm.

After the microplate was read and the absorbance values were determined the concentration values for the controls and samples of OPG (pmol/L) were determined from the calibration curve using a second degree polynomial equation.

After the absorbance values were determined, a standard curve was produced using the calibrator points. The concentration values for the controls and samples of OPG, expressed in pmol/L, were then determined from the calibration curve using a third order polynomial equation, with an average r^2 value of 0.9994 (0.9986-0.9998). The ranges for the high and low controls were 12.8-19.2pmol/L and 4.4-6.6pmol/L. All of the control values for each of the OPG ELISA kits completed fell within the ranges with an average of 18pmo/L for the high control and 5.9pmol/L for the low control. The intra-assay CV's were 5.7%, 9.1%, 3.9% and 4.3%, averaging 5.7% for all four microplates. The average inter-assay CV for 4 plates was 3.2% respectively.

N-telopeptides of type I collagen (NTx) was assayed using Osteomark® NTx Serum kits (Alere Scarborough, Inc., Scarborough, ME, USA). Prior to the commencement of the assay procedure the Working Strength Wash Solution, the Working Strength Conjugate Solution and the Calibrators, Controls and samples were all prepared. The Working Strength Wash Solution was prepared using 1 part 30x Wash Concentrate with 29 parts deionized water and was mixed using the magnetic stirrer for a minimum of 5 minutes. The working strength conjugate solution was prepared by

diluting 120µl of the Antibody Conjugate Concentration with 12ml of Antibody Conjugate Diluent. The solution was then mixed gently by inversion only. The Calibrators, Controls and samples were diluted 1:5, but because the childrens' values would fall above the standard curve their samples needed to be further diluted to 1:3 (van der Sluis, Hop, van Leeuwen, Pols, & de Muinck Keizer-Schrama, 2002). All of the diluted samples were mixed thoroughly using the vortex.

Following these preparations 100µl of each diluted Calibrator, Control and sample was pipetted into the microplate according to the plate configuration. Using a multichannel pipette, 100µl of the Working Strength Conjugate Solution was added to each well. A plate sealer was then applied and the microplate gently swirled on a flat surface for 15-20 seconds to ensure mixing. The microplate was incubated for 90 ± 5 minutes at room temperature. During the last 5 minutes of incubation the Chromagen Reagent/Buffered Substrate solution was prepared using a 1:101 of the Chromagen Reagent into the Buffered Substrate. The solution was mixed by inversion only. At the end of the incubation period the microplate was washed 5 times with the working strength wash solution and was blotted on absorbent paper after the final wash. Without delay 200µl of the diluted Chromagen Reagent/Buffered Substrate was added to each well using a multichannel pipette. A new plate sealer was applied and the microplate was then incubated for 30 ± 2 minutes at room temperature. During this time a blue colour will develop in the wells containing bound antibody-horseradish peroxidase conjugate. After the incubation period 100µl of the Stopping Reagent was added to each well, during which time the wells that developed a blue colour now turned yellow. The microplate was then swirled for 15-20 seconds on a flat surface and incubated for 5

minutes at room temperature before reading the absorbance values. Within 30 minutes of adding the Stopping Reagent the microplate absorbance values were read using a microwell plate reader that was set at 450nm.

The assay results were valid if the following criteria were met: (1) mean absorbance of the 0 nanomoles Bone Collagen Equivalents (nM BCE) Calibrator is greater than or equal to 1.300, (2) the span of the calibrator curve (absorbance difference between 0 nM BCE Calibrator and the 40 nM BCE Calibrator) is greater than or equal to 0.900, (3) the coefficient of variation (% CV) between concentration value (nM BCE) duplicates is ≤20% CV otherwise those > 20% CV should be rerun and (4) specimens giving absorbance values below the 40 nM BCE Calibrator should be diluted and retested.

After the absorbance values were determined, the concentration values for the controls and samples of NTx, expressed in nanomoles BCE/L (nM BCE), were determined from the calibration curve using a third order polynomial equation. The average r^2 was 0.995 (0.9862-0.9998). The childrens' concentration values were multiplied by their dilution factor of 3x. All of the mean absorbencies for the OnM BCE Calibrator were above 1.300 and the differences between the 0nM BCE and 40nM BCE above 0.900, which validates the results of the assays. In addition, all of the control values for the NTx ELISA kits fell within the low and high ranges of 6.1-10.3nM BCE. The intra-assay CV's were 2.8%, 2.4%, 2.7% and 3.1%, with an average of 2.8% and the average inter-assay CV for 4 plates was 4.9%.

Receptor activator of nuclear factor κB ligand (RANKL) was assayed using Human sRANKL (TOTAL) ELISA kits (BioVendor Research and Diagnostic Products, Candler, NC, USA). Prior to the assay procedure the standards, controls, Wash Solution Concentrate and samples were all prepared. The lyophilized Master Standard was reconstituted with Dilution Buffer and was left to dissolve for at least fifteen minutes with occasional gentle shaking. The set of standards was prepared using the amount of Dilution Buffer stated in the product data sheet. The lyophilized Quality Controls were reconstituted with deionized water and were left to dissolve for at least fifteen minutes with occasional gentle shaking. The Wash Solution Concentrate was prepared by diluting it 10x or ten-fold in deionized water for a 1x working solution. Samples were diluted 100x with Dilution Buffer and mixed thoroughly using the vortex.

Following the preparatory steps, 100µl of the Standards, Quality Controls and diluted samples were pipetted into the appropriate wells. A plate sealer was applied to the microplate, which was then incubated at 2-8 °C for 16-20 hours, shaking at 300rpm on an orbital microplate shaker. At the end of the incubation period the wells were washed 5 times using the Wash solution. After the final wash, the microplate was inverted and tapped strongly against paper towel. Using a multichannel pipette, 100µl of the Biotin Labelled Antibody was added to each well and was then incubated for an hour at room temperature, shaking at 300rpm on an orbital plate shaker. Following this incubation period, the wells were washed 5 times using the Wash Solution. After the final wash, the microplate was inverted and tapped strongly against paper towel. Afterwards, 100µl of Streptavidin-HRP Conjugate was added to each well using the multichannel pipette. A new plate sealer was applied and the microplate was then incubated for another hour at

room temperature, shaking at 300rpm on an orbital microplate shaker. At the end of the incubation period, the wells were washed 5 times for the final time using the Wash Solution. After the final wash, the microplate was inverted and tapped strongly against paper towel. Using a multichannel pipette 100µl of the Substrate Solution was distributed to each well. The microplate was then covered with a plate sealer as well as aluminum foil to avoid exposure to direct sunlight. It was incubated for 25 minutes at room temperature and was not to be shaken. During this step a blue colour should have formed and to stop the colour development 100µl of Stop Solution was added to each well using the multichannel pipette. Within 5 minutes of adding the Stop Solution absorbance was read using a microwell reader at 450nm.

Once the absorbance values were established, a standard curve was formed using the calibrator points. From the established curve, the concentration values of the controls and samples, expressed in pmol/L, were determined by extrapolating the values through a third order polynomial equation, with an average r^2 value of 0.9992 (0.999-0.9995). The measured concentrations of samples, calculated from the standard curve were multiplied by their respective dilution factor of 100x as they were diluted prior to the onset of the assay. The ranges of the low and high controls set by the kit were 3.9-5.8pmol/L and 9.4-14.0pmol/L. All of the controls fit within these required ranges. The intra-assay CV's were 11.3%, 6.4%, 4.5%, 7.9% and 6.7%, with an average of 7.4% and the average inter-assay CV for 5 plates was 9.1%.

3.4 Statistical Analysis

Statistical analyses were performed using SPSS version 21.0 for Windows. A paired t-test was used to examine group differences in physical characteristics, habitual physical activity, nutritional intake, time spent in each exercise intensity level, exercising heart rates and baseline measures of bone markers. A two-way analysis of variance (ANOVA) for repeated measures (RM ANOVA) (between-subject effect: group, within-subject effect: time) was used to assess group differences over time for the biochemical bone turnover markers. Note that a blood sample could not be obtained for one child at 1 hr post-exercise and for another child at both the 1 hr and 24 hr post-exercise time point (i.e. missing values). For these participants, the group's mean value at each time point was used for the purposes of the RM ANOVA analyses. In all cases assumption of sphericity was examined. In cases where sphericity could not be assumed the Greenhouse-Geisser test of significance was used. These cases are indicated in the Results section, where applicable. Bivariate correlations were examined using Pearson Product Moment Correlations. Data are reported as means and standard errors. Significance was assumed at an alpha level of 0.05.

Chapter 4 Results

4.1 Descriptives

A total of 30 participants completed the study, 12 of whom were pre- (n=7) and early (n=5) pubertal boys and 18 were adult men. Each group's mean age and anthropometric measurements, as well as the years from PHV and pubertal stage, are presented in Table 6. As expected, men were older and larger than boys (P<0.001), with no difference in adiposity between groups. The distribution of the sexual maturity levels (pubertal stage) in boys is shown in Table 7.

Table 6. Physical characteristics, body composition for healthy men and boys, as well as the boy's pubertal staging.

	Men (n=18)	Boys (n=12)	p-value
Age (yrs)	22.5 ± 0.7	10.2 ± 0.4	<0.001*
Height (cm)	180.1 ± 1.6	142.6 ± 2.0	<0.001*
Body Mass (kg)	78.6 ± 2.6	34.4 ± 1.5	<0.001*
BMI	24.1 ± 0.5	16.9 ± 0.6	<0.001*
PBF (%)	15.5 ± 1.5	13.4 ± 1.1	NS
LBM (kg)	66.0 ± 1.8	30.2 ± 1.2	<0.001*
Years to PHV	-	-3.05 ± 0.3	-
Pubertal Stage PH	-	1.67 ± 0.3	-

All values are expressed as means ± SE. Significance was set at p<0.05 difference between groups.
BMI = Body mass index, PBF = percent body fat, LBM = lean body mass, PHV = peak height velocity, PH = pubic hair

Table 7. Pubertal stage frequency table for boys (n=12)

Pubertal Stage PH	Boys (n=12)
1	7
2	2
3	3

The number of children in pubertal stages 1, 2 and 3 respectively
PH = pubic hair

4.2 Physical Activity

Table 8 describes the habitual physical activity of the two groups. No group differences were observed in physical activity scores, as assessed by the Godin-Shephard questionnaire or the PYPAQ ($p>0.05$), both of which assess general physical activity. However, using a bone-specific questionnaire (BPAQ), children demonstrated higher scores for both current physical activity and impact frequency scores ($p<0.02$, $p<0.01$), whereas adults demonstrated higher past activity scores ($p<0.01$).

Table 8. Physical activity scores, weekly physical activity metabolic equivalents, current and past scores and impact exercise frequency for healthy men and boys.

	Men (n=18)	**Boys (n=12)**	**p-value**
Strenuous (times/week)	4.6 ± 0.7	5.3 ± 1.1	NS
Moderate (times/week)	4.3 ± 0.8	3.3 ± 0.6	NS
Mild (times/week)	5.6 ± 0.4	4.3 ± 0.8	NS
Total Leisure Time PA (MET hours/week)	78.8 ± 8.7	77.3 ± 9.0	NS
PYPAQ Total (MET hours/week)	64.9 ± 16.9	58.5 ± 7.5	NS
BPAQ – Current	8.9 ± 1.4	18.7 ± 4.2	<0.02*
BPAQ – Past	85.3 ± 12.7	40.2 ± 5.8	<0.01*
BPAQ – Impact Frequency (times/week)	6.2 ± 0.8	10.2 ± 1.4	<0.01*

All values are expressed as means ± SE. Significance was set at $p<0.05$ difference between groups.
PYPAQ – Past Year Physical Activity Questionnaire, **BPAQ** – Bone-specific Physical Activity Questionnaire

4.3 Nutritional Intake

Table 9 presents the total daily intake of calories, calcium, vitamin D, caffeine, carbohydrates, fat and protein for the men and the boys, as assessed by the 24-hour nutrition recall interview. The two groups were similar in terms of their calcium and vitamin D intake. However, boys had a higher daily energy intake when corrected for

body mass differences ($p<0.006$). Caffeine intake neared significance with men showing a trend towards higher caffeine consumption ($p<0.06$). Men had significantly higher intakes of both fat and protein consumption in comparison to the boys ($p<0.02$, $p<0.001$).

Table 9. Total daily energy intake, total dietary consumption of calcium, vitamin D, caffeine, CHO, fat and protein for healthy men and boys (n=30).

	Men (n=18)	RDA	Boys (n=12)	RDA	p-value
Total Energy Intake (kcal)	2775 ± 229	3225	1888 ± 116	2266	<0.006*
Total Energy Intake (kcal/kg)	36.8 ± 3.7	41	56.4 ± 4.5	65.9	<0.02*
Calcium (mg)	1213 ± 144	1000	895.1 ± 90.5	1300	NS
Vitamin D (IU)	231.2 ± 46.1	600	200.2 ± 34.7	600	NS
Caffeine (mg)	46.3 ± 19.1	ND	1.0 ± 0.3	ND	NS
CHO (g)	354 ± 29.7	130	281 ± 17.0	130	NS
CHO (g/kg)	4.7 ± 0.5	ND	8.5 ± 2.8	ND	<0.001*
Fat (g)	99 ± 12.2	ND	58.6 ± 7.7	ND	<0.02*
Fat (g/kg)	1.3 ± 0.8	ND	1.7 ± 0.8	ND	NS
Protein (g)	131 ± 10.5	56	68.5 ± 5.3	34	<0.001*
Protein (g/kg)	1.7 ± 0.7	0.8	2.1 ± 0.7	0.95	NS

All values are expressed as means ± SE. Significance was set at $p<0.05$ difference between groups.
CHO – carbohydrates, **RDA** – recommended dietary allowance, **ND** – not determined. RDA were taken from (Health Canada, 2010)

4.4 Bone SOS

Bone SOS and Z-scores are presented in Table 10. As expected, men has significantly higher SOS scores for both dominant and non-dominant radius and tibia ($p<0.001$). There was also a significant difference in the non-dominant tibia Z-score ($P<0.05$). No group differences were observed in the radii Z scores and in the dominant

tibia Z score, but boys had a higher non-dominant tibial Z score. Importantly, all mean Z scores were between -1 and +1, indicating that bone properties were relatively close to the available norms for both age groups.

Table 10. Participant SOS (m/s) and Z-scores from the QUS measurement for dominant limbs for healthy men and boys.

	Men (n=18)	**Boys (n=12)**	**p-value**
Dominant Radius SOS (m/s)	4099 ± 25.2	3772 ± 19.4	<0.001*
Dominant Radius Z-Score	0.4 ± 0.2	-0.1 ± 0.2	NS
Dominant Tibia SOS (m/s)	3980 ± 20.4	3711 ± 22.4	<0.001*
Dominant Tibia Z-Score	0.04 ± 0.2	0.5 ± 0.3	NS

All values are expressed as means ± SE. Significance was set at $p<0.05$ difference between groups.
SOS – speed of sound

4.5 Exercise Protocol Measurements

The total exercise time for the men was 27.5± 1.5 min while for the children was 21.4 ± 1.0 min, ($p<0.004$). The exercising heart rates for each exercise at the completion of the required repetitions and for each of the three sets showed no significant differences between the two groups. It is evident that the exercise protocol was of high enough intensity to produce heart rates of at least 80% of the age-predicted maximum heart rates. The exercising heart rates at the completion of each set showed no significant differences between the two groups (Mean post-set heart rate: men: 172 ± 1 bpm; boys: 178 ± 1 bpm). The exercise protocol was of high intensity, resulting in heart rates ≥80% of the age-predicted maximum heart rates.

The accelerometery data revealed that, as expected, both groups experienced higher accelerations in the vertical axis than in the horizontal axis during exercise,

confirming that the majority of the exercise-related impact was in the vertical axis. Men had 6718 ± 581 counts per minute (CPM) in the vertical axis and the boys had 5356 ± 736 CPM in the vertical axis, with no significant differences between groups. Presented in Table 11 are the cut points thresholds between exercise intensities, based on the Freedson Adult (1998) and Freedson Children (2005) equations. For both groups, the mean CPM in the vertical axis was within the vigorous exercise intensity. Whereas the horizontal CPM's for both groups (men: 3247 ± 327 CPM; boys: 3088 ± 351 CPM) fell within the moderate intensity range, verifying that the exercise protocol was indeed vertically focused. The Cut Points in Table 13 are based on various activities such as walking, running and jumping (P. Freedson, Pober, & Janz, 2005; P. S. Freedson, Melanson, & Sirard, 1998).

Table 11. Freedson Adult (1998) and Freedson Children (2005) Cut Points (CPM)

	Sedentary	Light	Lifestyle	Moderate	Vigorous	Very Vigorous
Freedson Adult (1998)	0 – 99	100 – 759	760 – 1951	1952 – 5724	5725 – 9498	9499 – ∞
Freedson Children (2005)	0 – 149	150 – 499	NA	500 – 3999	4000 – 7599	7600 – ∞

CPM – counts per minute

4.6 Bone Turnover Markers

4.6.1 Baseline Measurements

Table 12 depicts the baseline values for each bone marker measured; BAP, NTx, OPG and RANKL, in both adult males and pre-pubescent boys. Both BAP and NTx

concentrations were significantly higher in the children. The OPG and RANKL concentration were similar in the two groups.

Table 12. Baseline values for BAP, NTx, OPG and RANKL in healthy men and boys

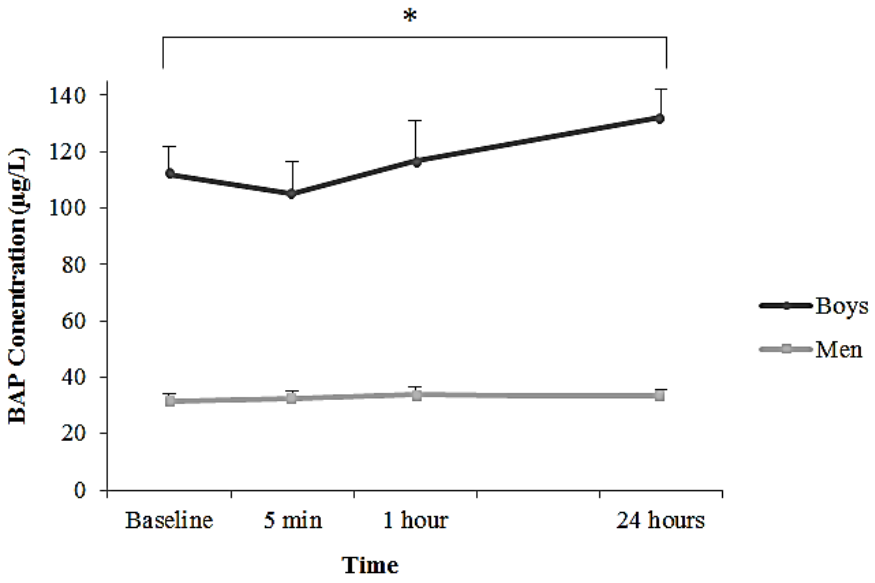
	Adults (n=18)	Children (n=10)	p-value
BAP (µg/L)	31.4 ± 2.6	112 ± 8.4	<0.001*
OPG (pmol/L)	3.4 ± 0.2	3.7 ± 0.3	NS
NTx (nM BCE)	22.5 ± 1.3	52.0 ± 3.9	<0.001*
RANKL (pmol/L)	322 ± 35.9	317 ± 51.1	NS

All values are expressed as means ± SE. Significance was set at p<0.05 difference between groups.
BAP – Bone-specific alkaline phosphatase, **NTx** – N-telopeptides of type I collagen, **OPG** – Osteoprotegerin, **RANKL** – Receptor activator of nuclear factor κB ligand

4.6.2 Bone Formation Markers – Effect of Exercise Session

4.6.2.a Bone-specific Alkaline Phosphatase (BAP)

BAP was significantly higher in children compared with adults at all of the time points (Group effect, $p<0.001$) (Figure 2). The RM ANOVA analysis revealed that sphericity could not be assumed ($p < 0.001$). Therefore, the Greenhouse-Geisser test of significance was used to examine the Time and Group-by-Time interaction effects (within-subject effects). The time effect was significant ($p=0.02$), reflecting an overall increase in BAP over time. The Group-by-Time interaction was significant ($p=0.04$), which reflects a higher increase 24 hr post-exercise in the boys compared with the men. Pairwise comparisons revealed significantly higher values 24 h post exercise, compared with pre-exercise values in boys only.



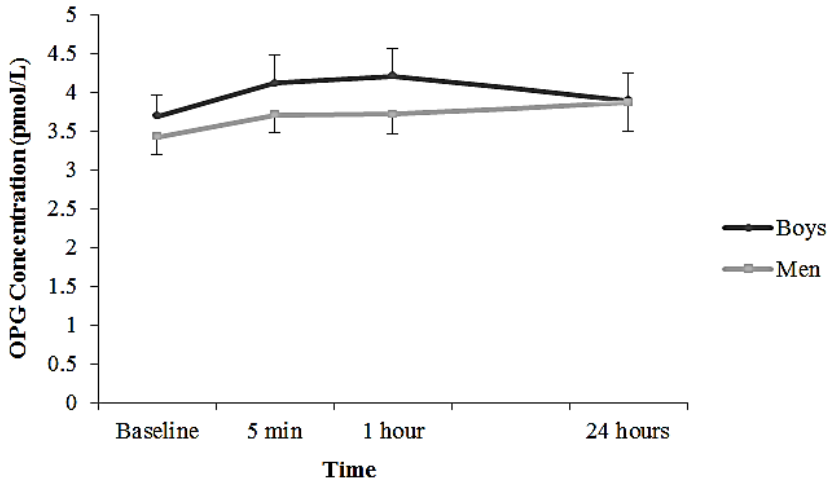
Sphericity $p < 0.001$
Group $p < 0.001$

	Greenhouse -Geisser
Time	$p = 0.04$
G x T	$p = 0.07$

Figure 2. BAP Concentrations (µg/L) throughout the different time points and their associated p-values. All BAP values are expressed as mean ± SE.
G x T – Group by Time, * = significant difference between pre- and 24 hr post-exercise in boys only.

4.6.2.b Osteoprotegerin (OPG)

The RM ANOVA showed that the OPG concentrations were similar across all time points in the men and boys (Group effect, $p<0.5$) (Figure 3). Because sphericity could not be assumed ($p<0.003$) the Greenhouse-Geisser test of significance was used to assess the Time and Group-by-Time interaction effects (within-subject effects). While there was a trend towards increased OPG values post exercise, neither the Time ($p<0.06$) nor the Group-by-Time interaction (within-subject effects) were significant ($p<0.05$).



Sphericity $p = 0.003$
Group $p = 0.4$

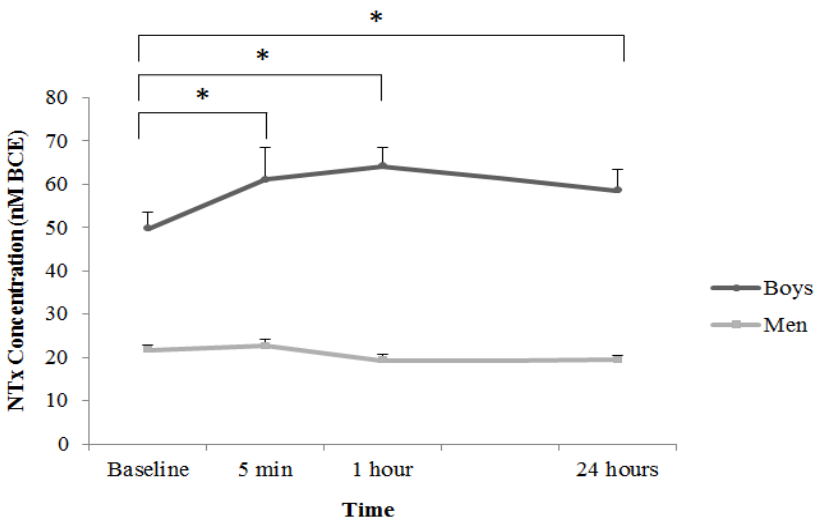
	Greenhouse -Geisser
Time	$p = 0.06$
G x T	$p = 0.5$

Figure 3. OPG Concentrations (pmol/L) throughout the different time points and their associated p-values. All OPG values are expressed as mean ± SE.
G x T – Group by Time

4.6.3 Bone Resorption Markers – Effect of Exercise Session

4.6.3.a N-telopeptides of type I collagen (NTx)

NTx concentrations were significantly higher in children in comparison to the adults throughout all four time points (Group effect, $p<0.001$) (Figure 4). The RM ANOVA disclosed that sphericity can be assumed ($p=0.3$). There was no Time effect but there was a significant Group-by-Time interaction ($p=0.03$), with a greater increase over time in boys than in men. Pairwise comparisons revealed significant increases in NTx at each time point post-exercise in boys only.



Sphericity $p = 0.3$
Group $p < 0.001$

	Sphericity Assumed
Time	$p = 0.1$
G x T	$p = 0.03$

Figure 4. NTx concentrations (nM BCE)) throughout the different time points and their associated p-values. All RANKL values are expressed as mean ± SE.
NTx – N-telopeptides of type I collagen**, G x T –** Group by Time, * = significant difference between time points.

4.6.3.b Receptor activator of nuclear factor κB ligand (RANKL)

The RM ANOVA revealed that RANKL concentrations were similar in both adults and children across all time points (Group effect, $p<0.6$) (Figure 5). Because sphericity could not be assumed ($p<0.001$) the Greenhouse-Geisser test of significance was implemented to assess the Time and Group-by-Time interaction effects (within-subject effects) throughout all four time points. There was no significant Time effect ($p<0.4$) or Group-by-Time interaction ($p<0.07$).

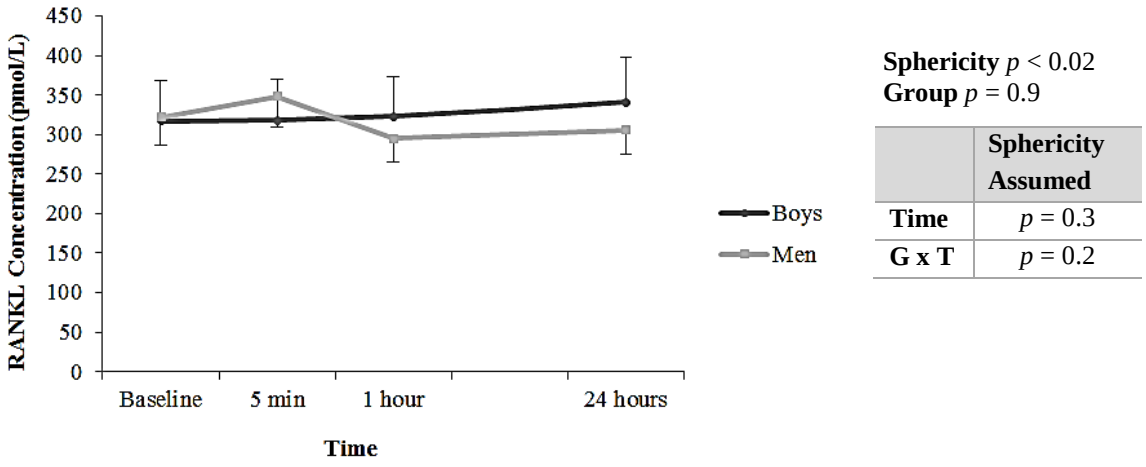


Figure 5. RANKL Concentrations (pmol/L) throughout the different time points and their associated p-values. All RANKL values are expressed as mean ± SD.
G x T – Group by Time

4.7 Habitual Physical Activity and Bone Turnover Response

Changes in bone turnover markers did not correlate with the reported physical activity in either the boys or the men.

4.8 Bone Markers at Different Pubertal Stages

Table 13 presents the baseline, 5 minutes post-, 1 hour post- and 24 hours post-exercise values of each bone marker for the boys of different pubertal stages. Because the sample size is so small, the results of the statistical analyses should be viewed with caution. For the purpose of the analysis, comparison was made between pre-pubertal (Tanner stage 1, n=7), and mid-pubertal boys (Tanner stage 2 and 3, n=5). A distinctly different pattern of response is not apparent between the maturity groups (Figures 6 – 9).

Table 13. Baseline, 5 minutes post-, 1 hour post- and 24 hours post-exercise values for boys of different pubertal stages. Tanner 1 (n=7), Tanner 2 (n=2) and Tanner 3 (n=3).

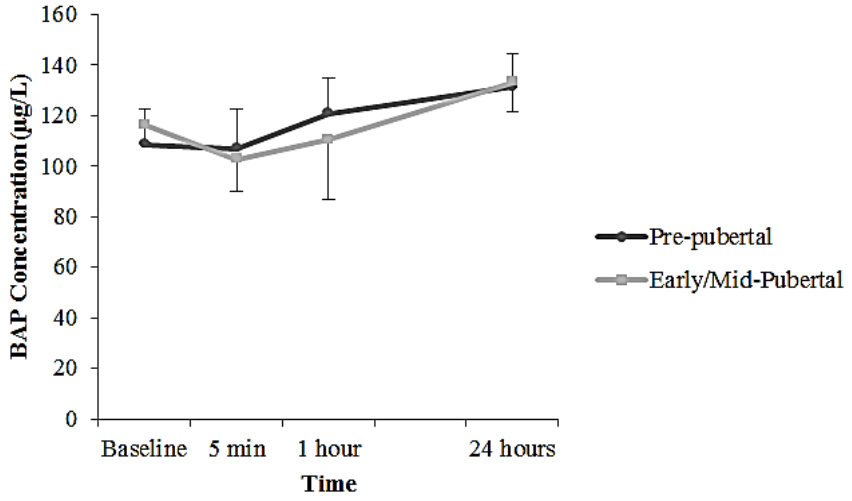
	Baseline	5 minutes	1 hour	24 hours
BAP (µg/L)				
Tanner 1	109 ± 14.0	107 ± 15.6	123 ± 13.9	131 ± 13.4
Tanner 2	111 ± 19.9	77.7 ± 20.1	145 ± 34.0	156 ± 18.7
Tanner 3	124 ± 4.6	150 ± 23.7	108 ± 43.2	140 ± 29.6
OPG (pmol/L)				
Tanner 1	3.7 ± 0.4	4.1 ± 0.5	4.1 ± 0.6	3.9 ± 0.5
Tanner 2	4.0 ± 0.03	4.7 ± 1.0	4.7 ± 0.08	4.5 ± 0.4
Tanner 3	3.6 ± 0.2	3.8 ± 0.2	4.0 ± 0.3	3.4 ± 0.4
NTx (nM BCE)				
Tanner 1	47.4 ± 5.8	62.3 ± 6.8	53.5 ± 3.2	47.7 ± 4.0
Tanner 2	44.5 ± 0.3	63.6 ± 49.0	89.8 ± 10.4	72.6 ± 13.5
Tanner 3	54.6 ± 9.0	55.4 ± 2.4	54.1 ± 12.3	72.0 ± 8.9
RANKL (pmol/L)				
Tanner 1	379.0 ± 55.2	403.0 ± 75.9	477.0 ± 73.1	444.0 ± 69.6
Tanner 2	59.7 ± 10.6	92.1 ± 30.7	77.7 ± 41.0	67.5 ± 26.1
Tanner 3	366.0 ± 97.9	388.0 ± 116	382.0 ± 132	449.0 ± 148

All values expressed as means ± SE.

BAP – Bone-specific alkaline phosphatase, **NTx** – N-telopeptides of type I collagen, **OPG** – Osteoprotegerin, **RANKL** – Receptor activator of nuclear factor κB ligand

4.8.1 Bone Formation Markers – Effect of exercise session at different pubertal stages

4.8.1.a Bone-specific Alkaline Phosphatase (BAP)

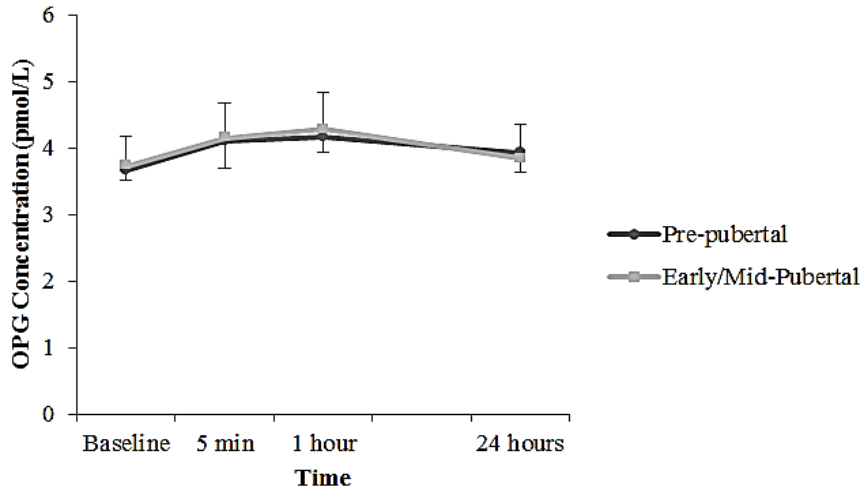


Sphericity $p < 0.3$
Group $p = 0.9$

	Sphericity Assumed
Time	$p = 0.1$
G x T	$p = 0.8$

Figure 6. BAP Concentrations (µg/L) throughout the different time points in pre- and early/mid-pubertal boys and their associated p-values. All BAP values are expressed as mean ± SE.
G x T – Group by Time

4.8.1.b Osteoprotegerin (OPG)



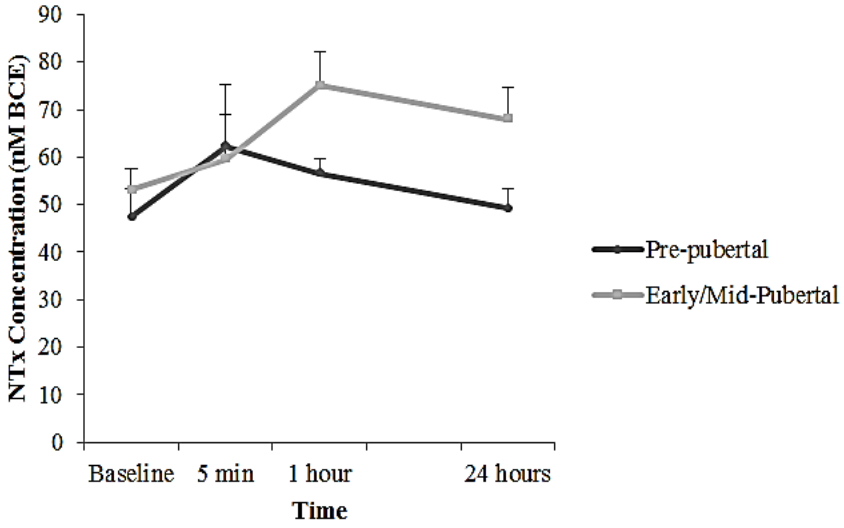
Sphericity $p < 0.8$
Group $p = 0.9$

	Sphericity Assumed
Time	$p = 0.03$
G x T	$p = 0.9$

Figure 7. OPG Concentrations (pmol/L) throughout the different time points in pre- and early/mid-puberal boys and their associated p-values. All OPG values are expressed as mean ± SE.
G x T – Group by Time

4.8.2 Bone Resorption Markers – Effect of exercise session at different pubertal stages

4.8.2.a N-telopeptides of type I collagen (NTx)

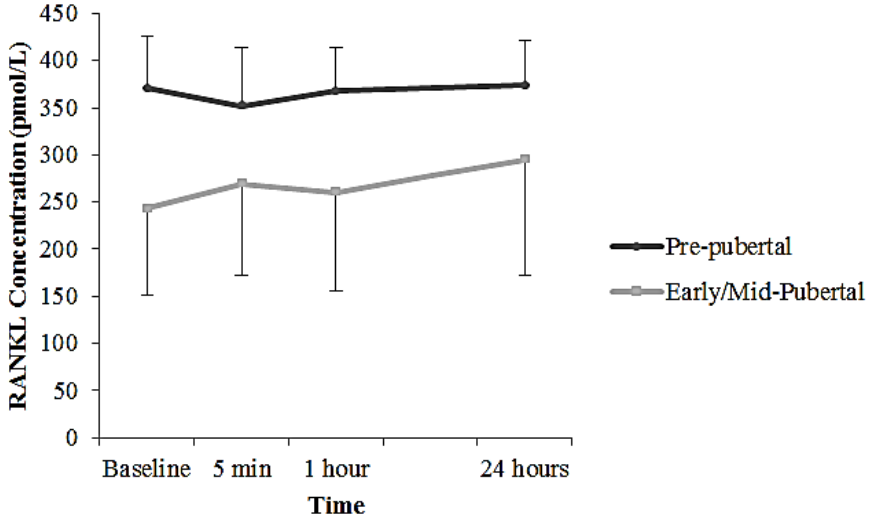


Sphericity $p < 0.8$
Group $p = 0.9$

	Sphericity Assumed
Time	$p = 0.2$
G x T	$p = 0.2$

Figure 8. NTx Concentrations (nM BCE) throughout the different time points in pre- and early/mid-pubertal boys and their associated p-values. All NTx values are expressed as mean ± SE.
G x T – Group by Time

4.8.2.b Receptor activator of nuclear factor κB ligand (RANKL)



Sphericity $p < 0.02$
Group $p = 0.7$

	Greenhouse -Geisser
Time	$p = 0.2$
G x T	$p = 0.1$

Figure 9. RANKL Concentrations (pmol/L) throughout the different time points in pre- and early/mid-pubertal boys and their associated p-values. All RANKL values are expressed as mean ± SE.
G x T – Group by Time.

Chapter 5 Discussion

5.1 Introduction

This is the first study to examine differences in the responses of markers of bone formation and bone resorption to an intense session of plyometric exercise between men and boys. The response was examined immediately after exercise, as well as 24 hours after the exercise session. The main findings of this study include: 1) Boys have greater concentrations of BAP (μg/L) and NTx (nM BCE) across all of the time points, whereas OPG and RANKL concentrations were similar in boys and men across all time points; 2) Impact exercise did not appear to affect the concentration of OPG and RANKL over a 24 hour time period in either boys nor men; and 3) BAP and NTx were significantly higher post-exercise in the boys but not in the men.

Neither the first or second hypotheses were confirmed in that serum levels of OPG and RANKL did not change following exercise. However, BAP and NTx were significantly increased after exercise in the boys, but not in the men. Thus, the pattern of change over time (third hypothesis) was similar in boys and men for OPG and RANKL, but not for BAP or NTx.

5.2 Habitual Physical Activity

When examining the results of the physical activity questionnaires, there were no significant group differences in Leisure-Time exercise and in past year PA. However, when looking at the BPAQ scores, boys had greater current scores but lower past scores compared with the men. The current PA results reflect the fact that children are generally more active than adults and the past PA results reflect the age factor in that the score is

cumulative. These results are in line with current literature (Caspersen, Pereira, & Curran, 2000; Livingstone, Robson, Wallace, & McKinley, 2003; Riddoch et al., 2007). However, it is surprising that the LTPA and PYPA scores were similar in the two age groups. One possible explanation is that the men's group comprised of individuals who were relatively active and therefore more inclined to volunteer for such a study. Indeed, the men's group included two elite athletes.

5.3 Nutritional Intake

The results of the 24 hour nutrition recall interview were used to assess intake of bone-related macronutrients and micronutrients including total energy, total relative energy, calcium, vitamin D, caffeine and the three macronutrients. Total energy intake was significantly higher in the adult group in comparison to the children. The average total energy intake fell below the estimated energy requirements for both groups (Health Canada, 2010). However, when body mass was taken into consideration the boys had higher total energy consumption, as expected, when compared to the men.

The recommended calcium intake of 1000mg for adults was exceeded in this study, whereas the children did not meet the recommended dietary intake of 1300mg. Neither group met the dietary requirement for Vitamin D intake (400IU or 10μg), although endogenous production was not measured or estimated. Vitamin D and calcium supplementation were inquired about through the subject screening and medical history questionnaire (Appendix 3.1). There were only two participants who reported vitamin D supplementation, one of which had just started two weeks prior to the test date. The supplements were included in the nutrition analysis. Vitamin D intakes for men were

similar to those reported in the literature. Rogers et al. (2010) reported a mean calcium intake of 1235 ± 219mg/day and vitamin D intake of 6 ± 2µg/day in untrained males between the ages of 24 and 62 years old (R. S. Rogers et al., 2011). Scott et al. (2010, 2011 and 2013) reported calcium intakes of greater than 700mg/day for all of the young recreationally active male participants (Scott et al., 2010; Scott et al., 2011; Scott et al., 2013). The boys, however, appear to have abnormally low values in comparison to the literature. Several studies have reported calcium intakes as low as 991 and as high as 1316mg/day which are higher than the values seen in this study (Falk et al., 2008; Falk et al., 2010). The vitamin D intake in the present study (5 ± 3µg/day) are similar to previously reported values in 10-16 year old children and adolescents (Falk et al., 2010). As would be expected, men consumed greater amounts of carbohydrates and protein in comparison to the boys. Both groups exceeded the recommended dietary allowance for carbohydrates and protein. The apparent high protein consumption of the men may reflect the fact that some of the men (n=4) consumed at least one protein shake daily. It is not clear whether the difference in nutritional intake between groups, specifically the fact that men's calcium consumption was above the RDA, while boys' consumption was below the RDA, affected the bone markers' response to the exercise session. No effects were apparent on bone properties (see below).

Unfortunately, food frequency questionnaires do not always reflect normal daily nutritional intake, especially when the data is based on one day (Rockett, Wolf, & Colditz, 1995). Nutritional questionnaires are often imprecise for capturing dietary intake; issues such as under-reporting do not allow for an accurate reflection.

5.4 Bone SOS

Bone SOS values were significantly higher in men in comparison to the boys, as expected. The bone SOS measurement served as an indicator of bone health status. This allowed us to ensure that there were no outliers with regards to bone strength. Despite group differences in daily calcium intake (relative to RDA), all of the participants' bone SOS values fell within normal ranges, as reflected by their Z-scores.

5.5 Exercise Measurements

It was evident that both groups were working at high intensity, as reflected by the high heart rates (≥80% of the age-predicted maximum heart rate) and accelerometry output. When looking at the time spent in moderate, vigorous or very vigorous activity, as a percentage of the total time spent exercising (not including resting periods); both groups spent the majority of their exercising time in those intensities. The accelerometer results indicate high intensity exercise, emphasizing the impact in the vertical axis. The Cut Points (CPM) developed by Freedson et al. (1998 and 2005) for adults and children, also confirm that both men and boys worked vigorously in the vertical axis as opposed to moderately in the horizontal axis (Turner & Robling, 2003). Therefore, it is assumed that the exercise protocol provided a potential osteogenic effect, as defined by others (P. Freedson et al., 2005; P. S. Freedson et al., 1998).

The adult group spent significantly more time completing the exercise protocol, which could be attributed to the recovery periods. All of the participants were allotted a minimum of 3 minutes recovery between sets. The adults typically exceeded the 3

minutes, whereas the children typically did not. Children have been shown to recover from short, high intensity exercise substantially faster than adults (Falk & Dotan, 2006).

5.6 Biochemical Markers of Bone Turnover

5.6.1 Baseline Values

As shown in Table 7, the boys in this study were pre- to mid-pubertal. Accordingly, the boys had a higher variability in the levels of bone markers, as reflected by the standard deviation values, for each measured bone marker in comparison to the men. This variability likely reflects the effects of growth and maturation. As documented, major fluctuations in bone markers occur at the onset of puberty, in accordance with the growth velocity or changes in secondary sexual characteristics (Hannon & Eastell, 2000; Szulc et al., 2000).

The baseline values of BAP for the children fell within previously reported values for healthy pre- and early-pubescent boys of 95μg/L to 114μg/L (Hui et al., 2003). Serum BAP reference ranges for men in the literature generally refer to older men than the men in this study. Additionally, serum BAP concentrations have been shown to decrease with age in older men (Hannon & Eastell, 2000). Therefore, it is not surprising that the men's baseline values were higher than the suggested values in the literature (6.8μg/L to 22.4μg/L) (Cavalier et al., 2010; Szulc, Garnero, Munoz, Marchand, & Delmas, 2001; Woitge, Scheidt-Nave et al., 1998).

The NTx baseline concentrations also fell within previously reported values of 50.6nM BCE to 71.7nM BCE for healthy pre- and early-pubertal (Tanner 1 and Tanner 2)

boys (van der Sluis et al., 2002). The only reference values available for serum NTx in adults are the ones provided in the package insert of the Osteomark® assay (6.2nM BCE to 19nM BCE) (Herrmann & Seibel, 2008). The mean NTx values for the men in this study were 22.5nM BCE, which is slightly higher than indicated by Herrman & Seibel (2008). However, the male reference range was conducted in men with an average age of 51 years, whereas the average age in this study was 22.5 years.

Because OPG and RANKL are relatively new bone markers, reference data in relation to age, sex and maturation has not been well established. The reported values for serum OPG in healthy children between the ages of 0.5 and 19 years of age ranges between 3.89 and 4.24pmol/L (Buzi et al., 2004; Gajewska, Ambroszkiewicz, & Laskowska-Klita, 2006; Wasilewska et al., 2009). The baseline values seen in the present study are at the lower end of these values at 3.7pmol/L for children. Kudlacek et al. (2003) examined the serum levels of OPG with increasing age in adults. The average level for men less than 30 years old was 4.9pmol/L and the values increased with increasing age. The average baseline value in this study was 3.4pmol/L. Vitamin D uptake has been shown to stimulate OPG production in vivo (Kudlacek et al., 2003). The participants did not reach their daily recommended intake for vitamin D intake, which might contribute to the lower serum OPG concentration. Since serum vitamin D, specifically 25-hydroxyvitamin, was not determined, their vitamin D status is not known.

In the present study, RANKL was analyzed as total RANKL, which is formed from the free and bound RANKL. Kerschan-Schindl et al. (2008) evaluated the serum levels of both free RANKL and total RANKL in healthy men between the ages of 42 and 50 years old. The values for these older men were between 41.5pmol/L and 236pmol/L.

The serum RANKL values seen in the adult group of this study were 322pmol/L, which appear to be much higher than the older adults. Serum levels of RANKL appear to decrease with increasing age, which may explain the difference between the two studies (Kerschan-Schindl et al., 2008). Representative values of total RANKL for children are not available in the literature.

5.6.2 Exercise Response

Boys displayed higher BAP levels with an increase 24 hours post-exercise (111.9 ± 29.2 vs. 137.6 ± 36.3µg/L, respectively), which was not observed in the men (31.4 ± 11.1 vs. 33.3 ± 10.5µg/L, respectively). NTx levels were higher in boys before and after exercise, with a greater increase over time in boys than in men (boys: 48.7 ± 13.7 vs. 58.8 ± 16.7nM BCE pre- and 24 hours post-exercise, respectively; men: 21.7 ± 5.4 vs. 19.4 ± 5.0nM BCE pre- and 24 hours post-exercise, respectively). OPG and RANKL levels were similar in boys and men before and after exercise, with no change over 24 hours.

Two previous studies examined the effect of a plyometric exercise session on bone turnover in adults, but not in children (Lin et al., 2012; R. S. Rogers et al., 2011). Lin et al. (2012) assessed the response of OC and TRAP5b acutely to plyometric jumping in young men. The exercise protocol consisted of approximately 150 jumps with an average heart rate of 164 bpm. Blood samples were collected before, 5 minutes, 1, 3, 6, 24, 48 and 72 hours post exercise trials (Lin et al., 2012). Rogers et al. (2011) assessed the response of BAP and TRAPb5 to a single session of plyometric jumping (approximately 130 jumps) in young and older men and blood samples were collected

before exercise, immediately following exercise, 15, 30 minutes, 1, 2 and 24 hours post-exercise (R. S. Rogers et al., 2011).

In terms of bone formation, the results of the two studies are inconsistent in that Lin et al. (2012) observed an increase in OC, while Rogers et al. (2011) observed no change in BAP levels after exercise. In the present study, no BAP changes were observed after exercise in the men, in agreement with Rogers et al. (2011). The latter argue that a possible increase in BAP levels may have been masked by the natural morning decrease in BAP (Table 3). This decrease typically occurs around 6:30am and our participants were tested between 9:00am and 9:30am. Unfortunately, we did not include a time-matched control session to confirm these assumptions. Nevertheless, taken together, it is suggested that high impact exercise induces an acute osteogenic effect in adults, although this effect may not be substantial.

In terms of bone resorption, TRAP5b did not change in one study (Lin et al. 2011) but decreased post-exercise in another study (Rogers et al. 2011). In the present study, no significant effect of exercise was observed on NTx or RANKL in the men. This is in line with Lin et al. (2012). The discrepancy between the present study (no change in bone resorption markers) and the results of Rogers et al. (2011) (decrease in bone resorption marker 15 and 30 min post-exercise) may possibly be due to the difference in sampling times between the two studies. The decrease in TRAP5b was observed 15 and 30 min post-exercise, but at 1 hour, values were not significantly different from pre-exercise. Similarly, in the present study, no difference was observed in bone resorption markers 1 hr post-exercise in the men. It is possible that some increase in bone resorption was 'missed' between 5 min post- and 1 hour post-exercise.

In terms of pediatric bone metabolism responses to single bouts of exercise, the research is limited to one study. Pomerants et al. (2008) examined the effect of acute exercise on serum markers of bone turnover in 60 healthy schoolboys, 10-18 years old. The boys underwent a 30 minute constant load, cycle ergometer bout at approximately 95% of their ventilatory threshold. The average heart rates achieved were 169bpm, which is ≥80% of the age-predicted maximal heart rate. The results revealed that although there was a slight increase 30 minutes post exercise in serum PINP and ICTP levels, markers of bone formation and resorption, respectively, it did not reach statistical significance. These results are in line with the fact that cycling does not provide weight bearing loads and therefore, may not provoke a sufficiently high osteogenic effect to induce significant effects on bone turnover. By contrast, in the present study, both BAP and NTx, markers of bone formation and resorption, respectively, increased following exercise in the boys. The inconsistency between the two studies likely reflects the different exercise modes (cycling vs. jumping – no-impact vs. high impact), which were used in the two studies.

The mechanical loads applied to bone generate mechanical signals at the cellular level, the nature of which is not known. What is known is that mechanical loads can stimulate a response from both osteoblasts and osteoclasts (Frost, 1997; Frost, 1999). It is evident that the mechanistic effects differ between the boys and men, as reflected by the increased BAP and NTx in the boys, induced by the plyometric exercise, with no effect in the men. Children's bones undergo greater turnover compared with adults (Szulc et al., 2000). This is supported by the higher baseline BAP and NTx values in the boys observed in this study. The higher turnover rate in the children may render their cellular bone activities more responsive to mechanical stimuli. Indeed, Frost (1997,1999)

described an age-related decline in bone cellular responsiveness in aging adults (Frost, 1997; Frost, 1999). Thus, the higher responsiveness may explain the greater increase in BAP and NTx markers after exercise in the boys. Notably, the mechanical stimulus in this study was designed to be similar in the boys and the men (similar number of jumps from size-adjusted height).

Although there was no clear immediate change post-exercise in bone markers in the men, it is known that exercise has a long-term maintenance effect on bone in adults. Therefore, the mechanism remains unclear.

Because OPG and RANKL are considered essential cytokines rather than enzymes or degradation products of bone resorption and formation, it may be possible that their response to exercise differs from that of BAP or NTx. It has been suggested that mechanical stimuli can directly affect osteoblasts and indirectly affect osteoclasts (Robling, Castillo, & Turner, 2006). The indirect osteoclastic response reduces the expression of RANKL and increases the expression of OPG, which in turn decreases the number of osteoclasts (Robling, Castillo, & Turner, 2006). However, the timeline of this process in response to exercise is not known. Ziegler et al. (2005) studied the effects of either a half marathon or full marathon on OPG and RANKL in recreational male and female middle-aged runners. In both groups, a significant decrease in RANKL was observed, whereas a significant increase in OPG was only apparent in the full marathon group. The results of this study suggest that the running distance and duration play an important role in OPG and RANKL changes (Ziegler et al., 2005). Although the exercise protocol in the present study involved very high mechanical stimuli, the duration was

relatively short. It is possible that multiple sessions or a larger duration of exercise would induce a change in OPG and RANKL values.

It has been suggested that acute exercise may not be sufficient to modify serum concentrations of markers of bone turnover (Banfi et al., 2010). In fact, the effects of exercise may not be apparent until 2 or 3 days post exercise. Scott et al. (2013) demonstrated that increases in serum OPG levels did not appear until 2 days post exercise of two 60 minute treadmill running bouts (Scott et al., 2013). In previous studies also done by Scott et al. in 2011 and 2010 there were also increases in bone metabolism markers following the 24 hour post exercise time period. When recreationally active young males completed a 60 minute running treadmill protocol increases in BAP were seen 3 and 4 days post exercise and decreases in OC 3 days post exercise (Scott et al., 2011). When recreationally active young males completed an intermittent exhaustive running protocol, increases in β-CTx were evident 1-4 days post exercise (Scott et al., 2010). Ashizawa et al. (1998) also demonstrated delayed bone marker responses with young, untrained males executing a resistance exercise protocol. BAP decreased 2 and 3 days post exercise (Ashizawa et al., 1998). Therefore, it is possible that the plyometric exercise in the present study induced changes in bone turnover markers in the men but that these changes would be evident after more than 24 hours. Although the exercise protocols described above are not plyometric, nor do they involve children, it is suggested that in the future, post-exercise blood samples should exceed the 24 hour time point as it may take several hours and up to several days to show changes (Banfi et al., 2010).

Chapter 6 Conclusion

6.1 General Conclusions

The present study aimed to compare the acute response of markers of bone turnover following a single session of plyometric exercise in adult males and young boys. The results demonstrated that children had higher levels of both BAP and NTx at baseline as well as throughout the post-exercise period. This was not the case with the levels of OPG and RANKL which were similar in adults and children. In addition, boys showed an increase in BAP and NTx 24 hours post-exercise, which was not seen in the men. Thus, it can be concluded that a single session of plyometric exercise may stimulate bone turnover, as reflected by the increase in both BAP and NTx levels, in boys but not in men suggesting that children are more responsive to the effects of plyometric exercise. This is in line with the previously described enhanced bone accretion rate during childhood and especially, during the early pubertal period (Malina, R.M., Bouchard, C. & Bar-Or, O., 2004; Seibel, 2002).

6.2 Implications

It is known that high impact exercise has the greatest benefits on bone but the timing of response is not well known or understood. From a methodological perspective, this information could aid researchers in determining appropriate timing to examine the effects of an intervention on bone turnover without contamination of a prior exercise session, The results of the present study indicate that 24 hours is an insufficient time period to exclude the effects of the last training session of a plyometric exercise protocol in boys. In men, no changes with time were evident. This may indicate that changes in

biochemical markers of bone turnover are apparent after a longer time period, or that these changes are more apparent on a different time line than the boys (e.g., possibly after 60 min but before 24 hrs). Further research is necessary to examine these possibilities.

Aside from the methodological perspective, a more practical approach involves the determination of whether measurement of serum bone markers is sensitive enough to detect changes in bone turnover markers following a single bout of exercise. There was an obvious osteogenic effect in the boys, as reflected by the increase in both BAP and NTx, which may suggest that their bone cellular activities are more responsive than men's to mechanical stimuli. The lack of an apparent effect in OPG and RANKL may indicate that the measurement of these markers in serum is not sensitive to the effects of exercise in boys or in men.

6.3 Strengths

The present study has numerous strengths. First, this study was the first to examine the acute response of bone markers to a plyometric exercise session in children. In addition, it was also the first to compare the acute response of serum markers of bone turnover in men and boys. The present study incorporated several questionnaires regarding habitual physical activity and nutritional intake as well as bone health status which provided insight into whether habitual physical activity or bone strength affects the bone turnover markers' response in boys or in men. The plyometric exercise protocol was designed to provide ground reaction forces high enough to induce an osteogenic effect, as reflected by the long-term changes in bone structure and mineralization. All participants

were tested consistently at the same time to minimize the circadian rhythm variation of the various bone markers.

6.4 Limitations

The participants in each of the groups were physically active to various degrees. None of the participants were competitive athletes but habitual physical activity and training varied considerably. It is possible that with a more homogenous group, the variability in our results would have been reduced, thus increasing the power to detect significant differences over time or between groups. Along these lines, the small sample size of the boys' group may have resulted in reduced power to detect significant group differences.

Additionally, all testing took place at the same time of day. However, we did not include a time-matched control session. This would have allowed us to take into account the circadian rhythms of the measured bone markers by following the same blood collection schedule without the exercise protocol in the same participants.

It is known that the majority of bone markers are present in other tissues aside from bone, and the measured levels may be influenced by non-skeletal properties. Therefore, it is important to note that the measured quantity of each bone marker may not be solely skeletally derived. Moreover, conclusions based on changes of bone marker concentrations alone may not be entirely accurate since the deviations may be a reflection of plasma volume shifts. Exercise is accompanied by hemoconcentration and during recovery plasma volume expansion occurs. Therefore, it is sometimes recommended to interpret the changes in bone marker concentrations alongside the changes in plasma

volume. This may result in a truer indication of the changes in bone metabolism (Fellmann, 1992). Brahm et al. (1997) studied the influence of plasma volume and physical fitness with regards to bone metabolism and exercise. Significant reductions in plasma volume during exercise were evident and there was a marked expansion following 24 hours of recovery. However, Rogers et al. (2011) who examined the acute response of markers of bone turnover to a single bout of plyometric exercise, also examined changes in plasma volume at all of the time points. These changes in plasma volume were minimal and the concentrations of the analytes did not need to be adjusted (R. S. Rogers et al., 2011).

Finally, plyometric exercise was used as a stimulus for bone turnover in the present study, assuming that the high impact of the jumping has a local effect. However, exercise may also have a systemic, whole-body effect, which may be better reflected in hormonal changes. Thus, one limitation of this study is that hormonal changes of GH, PTH. Testosterone, estrogen and IGF-1 were not examined, which would provide further information on the anabolic effects exercise exerts on bones and muscles.

6.3 Future Directions

In the present study, two markers of bone formation and two markers of bone resorption were examined before and after exercise. The pattern of response was dissimilar in the measured markers. That is, while one marker of bone formation (BAP) and one marker of bone resorption (NTx) increased, in the boys, the other markers did not. Thus, the mechanism by which exercise affects bone metabolism is still unclear. Future studies should examine the local and systemic effects that exercise has on bone

turnover. For example, numerous hormones may affect bone turnover. These hormones generally have a systemic effect. However, the mechanical effect of plyometric or high-impact exercise presumably has a local effect. The interaction between the systemic (hormonal) and local (mechanical) effects on bone turnover needs to be further examined in children and adults, males and females. This can be achieved by measuring the baseline values of the aforementioned hormones and their response to an exercise protocol such as the one used in the present study as well as in response to an exercise protocol which does not involve high impact (e.g., cycling, swimming).

A different response pattern was observed between children and adults. Future studies should examine children of different ages and especially, of different maturity status, in order to elucidate the period during which children's response becomes more adult-like.

In the present study, BAP and NTx were elevated 24 h after exercise in the boys. Future studies are important to determine the timeline for the return of levels to resting values. That is, future studies are needed to examine the response of these bone turnover markers over a period longer than 24 hours.

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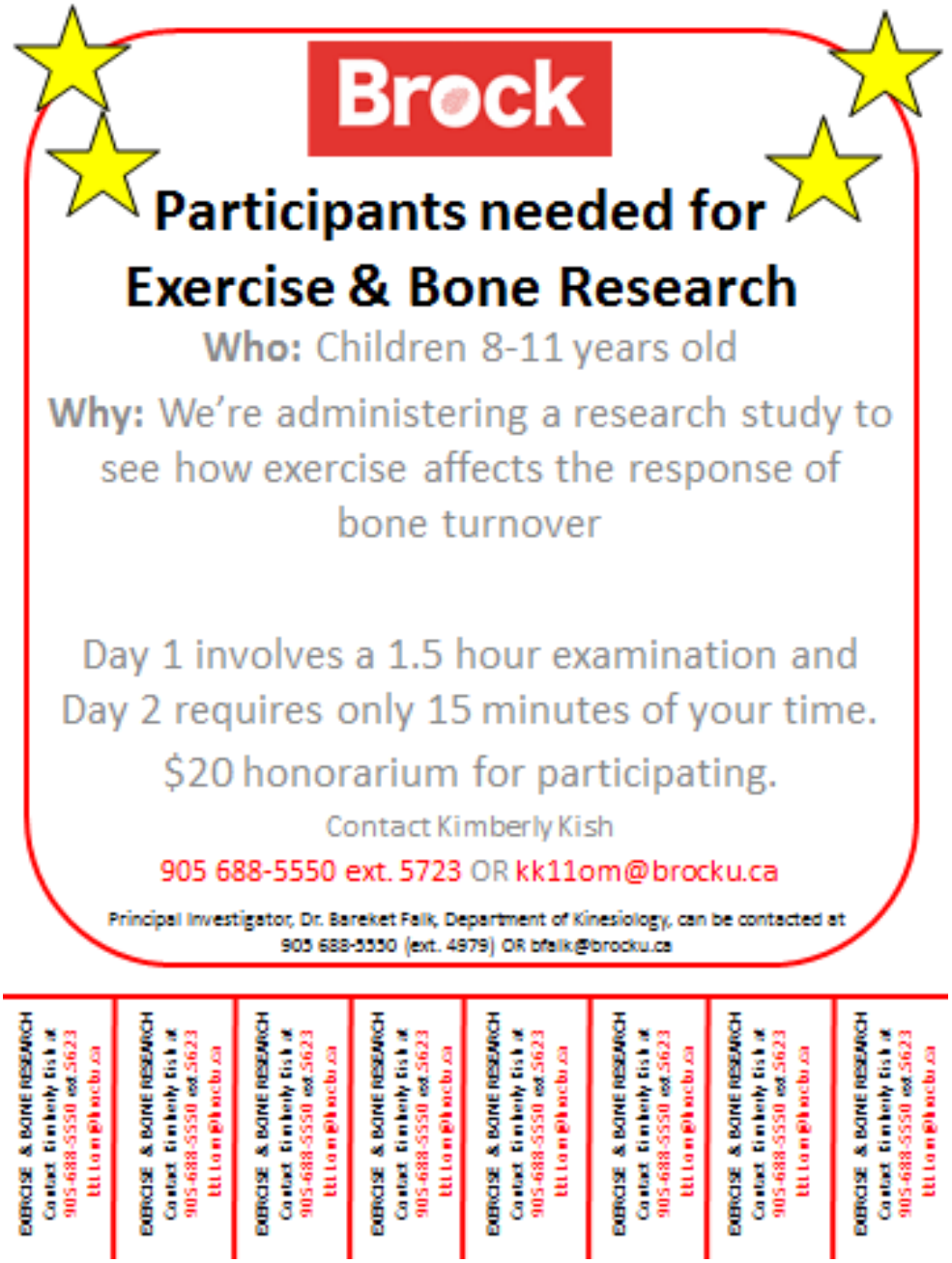
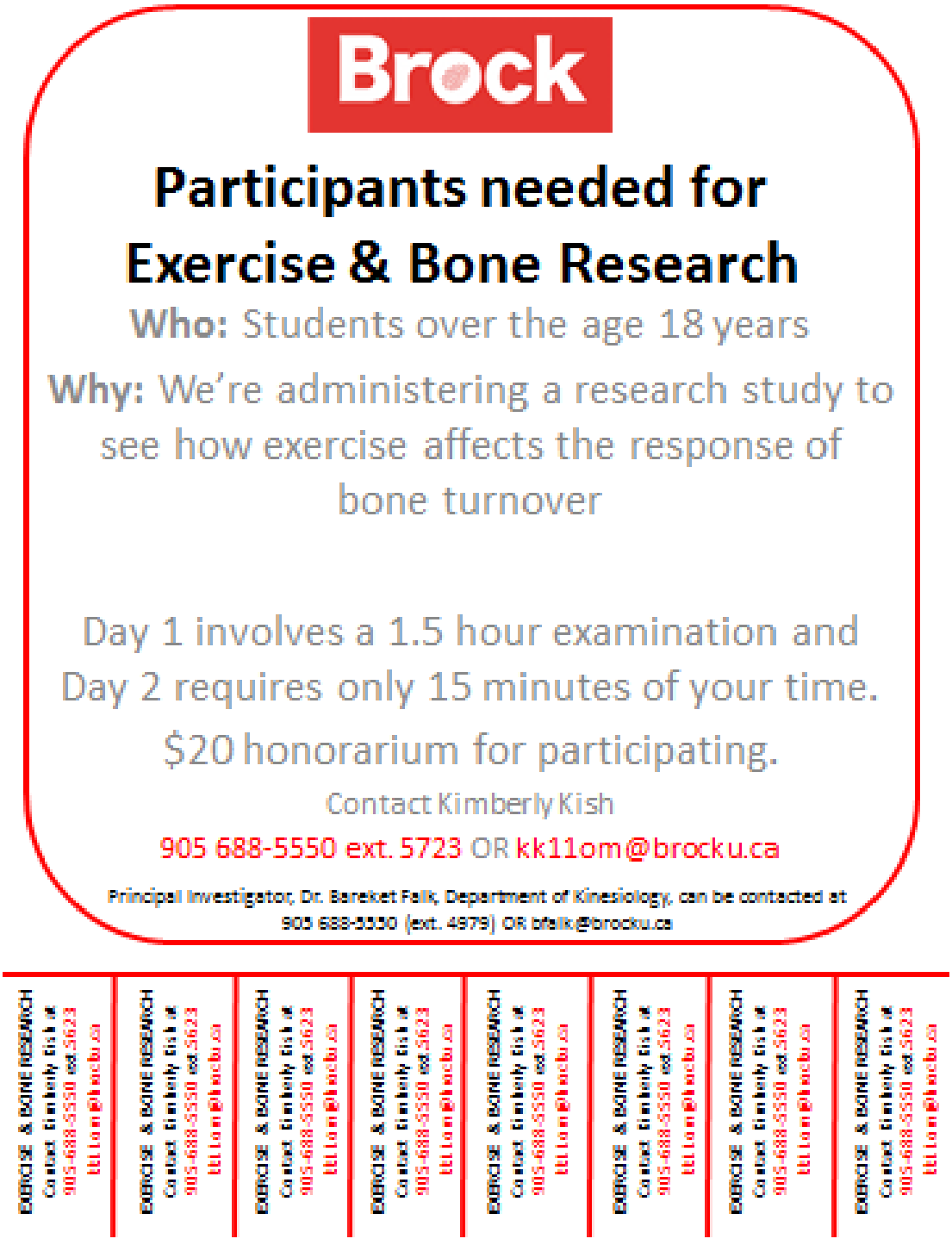
Appendix 1 Recruitment

1.1 Posters

1.1.1 Recruitment Poster for Children



1.1.2 Recruitment Poster for Adults



1.2 Newspaper Advertisement

WHO: Boys 8 - 11 yrs old
WHY: To determine the effect of exercise on bone turnover
WHAT IS INVOLVED: Two visits to Brock University (1.5 hr + 30 min)
An exercise session and blood samples before and after exercise.
$20 honorarium for participating.
CONTACT: Kimberly Kish
905 688-5550 ext. 5623 OR
kk11om@brocku.ca

This study has been reviewed and received clearance from the Brock University Research Ethics Board (file # 12-051)
reb@brocku.ca, 905-688-5550 ext 5623t.
Principal Investigator, Dr. Bareket Falk,
Department of Kinesiology, can be contacted at
905 688-5550 (ext. 4979) OR
bfalk@brocku.ca

1.3 Letters of Invitation

1.3.1 Letter of Invitation for Children

Invitation Letter

Bone Turnover Following Exercise in Children and Adults

Principal Investigator: Dr. Bareket Falk, Department of Physical Education and Kinesiology, Brock University

Principal Student Investigator: Kimberly Kish, Msc. Candidate, Department of Kinesiology, Brock University

If you have a male child between the ages of 8 and 11 years, we would like to invite you and your child(ren) to participate in this study.

The **purpose** of this research project is to investigate the acute response and recovery of bone metabolism induced by a variety of jumping exercises over a period of 24 hours in children and adults.

Tests and measurements will require approximately **1.5 hours** on one day and **15 minutes** on the post-exercise day. Briefly, measurements include filling out several questionnaires, completing a 30 minute exercise routine, measurements of bone turnover (using blood samples) and bone strength (using ultrasound).

Participation in this project will provide information on your child's bone strength, as well as other information, such as height, weight and percent body fat.

There is a $20 honorarium for participating.

This research is being performed by Brock University researchers in the Applied Physiology Laboratory

If you are interested in participation or if you would like some more information, please fill out the form on the next page and return it in the enclosed envelope. You can also e-mail the requested information to Kim Kish (Project Coordinator) at kk11om@brocku.ca, **or to Bareket Falk (Principal Investigator) at** bfalk@brocku.ca**.**

If you have any pertinent questions about your rights as a research participant, please contact the Brock University Research Ethics Officer (905 688-5550 ext 3035, reb@brocku.ca)

Thank you,

Dr. Bareket Falk and Kimberly Kish
Department of Physical Education and Kinesiology
Faculty of Applied Health Science
Brock University
Tel: 905-688-5550 ext: 4979 or 5623
Email: bfalk@brocku.ca or kk11om@brocku.ca

If you are willing to be contacted to discuss having your child participate in this study, please complete one of the two following options:

OPTION 1: Fill out the information below and return this form in the enclosed envelope

OPTION 2: Send the information below to Kim Kish (Project Coordinator) at kk11om@brocku.ca

Child's first and last name:

_____

Child's age: _____

Child's sex: _____

Parent's first and last name:

_____

Please contact me at:

_____

At the following days and times:

1.3.2 Letter of Invitation for Adults

Invitation Letter

Bone Turnover Following Exercise in Children and Adults

Principal Investigator: Dr. Bareket Falk, Department of Physical Education and Kinesiology, Brock University

Principal Student Investigator: Kimberly Kish, Msc. Candidate, Department of Kinesiology, Brock University

We would like to invite you to participate in the present study, which investigates bone turnover following an acute bout of exercise.

The **purpose** of this research project is to investigate the acute response and recovery of bone metabolism induced by a variety of jumping exercises over a period of 24 hours in children and adults.

Tests and measurements will require approximately **1.5 hours** on one day and **30 minutes** on the post-exercise day. Briefly, measurements include filling out several questionnaires, completing a 30 minute exercise routine, measurements of bone turnover (using blood samples) and bone strength (using ultrasound).

Participation in this project will allow you to have personal information on your bone strength as well as other information, such as height, weight and percent body fat.

This research is being performed by Brock University researchers in the Applied Physiology Laboratory

If you have any pertinent questions about your rights as a research participant, please contact the Brock University Research Ethics Officer (905 688-5550 ext 3035, reb@brocku.ca)

If you are interested in finding out more about this study, please contact us by email (kk11om@brocku.ca, or bfalk@brocku.ca) or by phone (905-688-5550 ext: 4979 or 5623).

Thank you,

Principal Investigators:

Dr. Bareket Falk and Kimberly Kish
Department of Physical Education and Kinesiology
Faculty of Applied Health Science
Brock University
Tel: 905-688-5550 ext: 4979 or 5623
Email: bfalk@brocku.ca or kk11om@brocku.ca

Appendix 2 Consent Forms

2.1 Consent Form for Children

INFORMATION AND CONSENT TO PARTICIPATE IN RESEARCH

Exercise and Bone Turnover

You are being invited to participate in a research study being conducted by the investigators listed below. Prior to participating in this study please read this form to find out about the purpose and the tests of this study. For the tests you will have to visit the Exercise Physiology Laboratory (WH 23, Brock University). This study is part of the Faculty of Applied Health Sciences of Brock University.

INVESTIGATORS:	**DEPARTMENT:**	**CONTACT:**
Dr. Bareket Falk	FAHS, Brock University	(905) 688-5550 ex. 4979
Kimberly Kish	FAHS, Brock University	(905) 688-5550 ex. 5623

PURPOSE:

The purpose of the study is to investigate the acute response and recovery of bone metabolism induced by jumping exercise over a period of 24 hours in children and adults.

DESCRIPTION OF TESTING PROCEDURES:

If you agree to volunteer for this study, you will visit our laboratory for one session of testing, lasting approximately 1.5 hours and another session, 24 hours later, lasting approximately 30 min. At the end of the study, you will be given a summary of the findings, upon request. It is recommended that you come for the measurements in shorts and a short sleeved shirt. Parents may be present at all stages of the study.

Your child will undergo the measurements and procedures listed below; please note that in all questionnaires, you may choose not to answer any question.

A. Pre-exercise Assessments:

1. Your child will be asked to complete several questionnaires, outlining their medical history, physical activities, nutritional habits and pubertal status. The questionnaire used to measure pubertal status involves your child looking at drawings of genitalia and deciding which stage of puberty they best match. This will be carried out in a private room to avoid any uneasiness. Also, please be

aware that the medical history questionnaire includes questions about drug use, alcohol use and smoking. In all questionnaires, your child may choose not to answer any question without penalty.

2. Body Composition: we will measure your height, weight and percent body fat. Percent body fat will be estimated using skinfold thicknesses and bioelectrical impedance analysis (BIA). The BIA assessment requires your child to stand on a weight scale and grasp handles. A mild electrical current (50kHz, 800μA) will pass through your hands to your feet. This current cannot be felt and causes no harm. Valid measurements require abstinence from exercise, alcohol consumption, and eating/drinking for at least 12, 24 and 4 hours, respectively, prior to testing.

3. We will determine your child's bone strength using the Sunlight Omnisense™ ultrasound system. This procedure involves the application of gel to the forearms and the lower legs and moving an ultrasound probe over these regions. This procedure is quick and causes no discomfort.

4. Blood Analysis: A total of 4 blood samples will be collected to determine biochemical markers affecting bone turnover: pre-exercise, immediately after exercise, 60 min and 24 hours post exercise. The blood samples will be drawn using a standard venipuncture technique performed by a certified lab assistant. Up to 10ml will be withdrawn. It should be noted that the venous blood drawing procedure is a routine procedure performed by a certified technician and offers minimal risk to participants. In rare instances, participants may experience slight pain and/or tingling in the area and/or a minor bruise from the needle. However, with the use of anaesthetic creams (e.g., Emla), which we use in the laboratory, any sensation of pain is minimal.

B. Exericse Protocol:

Participants will begin with a warm-up activity that will consist of 5 minutes of low intensity jogging with a series of stretching exercises. The exercise protocol will be designed to provide high impact, weight-bearing loads in the form of circuit training stations. Participants will be instructed to rotate through the five stations, which will be composed of jumping jacks, lunge jumps hopping, jumping over various obstacles (ie, hurdles) and drop jumps, for a total of 3 sets. Each station will take 2 minutes and the participants will then be instructed to progress to the next one. A recovery period of 2 minutes will be given between each set.

CONFIDENTIALITY:

All data collected during this study will remain confidential and will be stored in offices and on secured computers to which only the principal and co-investigators have access. You should be aware that the results of this study will be made available to scientists, through publication in a scientific journal but your name and any personal data will not appear in compiling or publishing these results. Data will be kept for 5 years after the date of publication, at which time all information will be destroyed. Additionally, you will have access to your own data, as well as group data when it becomes available and if you are interested. This can be provided to you by simply contacting the principal investigators.

PARTICIPATION AND WITHDRAWL:

You and your child can choose whether to participate in this study or not and may remove your data from the study if you wish. Your child may also refuse to answer any questions posed to them during the study and still remain as a subject in the study. The investigators reserve the right to withdraw you from the study if they believe that it is necessary.

RISKS AND BENEFITS:

Participation will allow your child to become exposed to a research protocol, contribute to the advancement of science and, gain personal and general knowledge about their own body. All results will be provided to you upon request.

The only foreseeable risks involved in participation include:

a) Possible muscle soreness within 48 hours of the test. If this occurs, it will only be temporary.
b) In rare instances, participants may experience slight pain and/or tingling in the area and/or a minor bruise from the venous blood draw.

RIGHTS OF RESEARCH PARTICIPANTS:

You will receive a signed copy of this consent form. You may withdraw your consent to participate in this study at any time, and you may also discontinue participation at any time without penalty. In signing this consent form or in participating in this study you are not waiving any legal claims or remedies. This study has been reviewed and received clearance from the Brock University Research Ethics Board (file # 12-051) If you have any pertinent questions about your rights as a research participant, please contact the Brock University Research Ethics Officer (905 688-5550 ext 3035, reb@brocku.ca).

INFORMATION:

Please contact Dr. Bareket Falk at 905 688-5550 (ext. 4979), bfalk@brocku.ca or Kimberly Kish at 905 688-5550 (ext. 5623), kk11om@brocku.ca if you have any questions about the study.

I HAVE READ AND UNDERSTAND THE ABOVE EXPLANATION OF THE PURPOSE AND PROCEDURES OF THE PROJECT. I HAVE ALSO RECEIVED A SIGNED COPY OF THE INFORMATION AND CONSENT FORM. MY QUESTIONS HAVE BEEN ANSWERED TO MY SATISFACTION AND I AGREE TO PARTICIPATE IN THIS STUDY.

SIGNATURE OF PARENT/GUARDIAN DATE

PRINTED NAME OF PARTICIPANT

WITNESS DATE

In my judgment the participant is voluntarily and knowingly giving informed consent and possesses the legal capacity to give informed consent and participate in this research study.

SIGNATURE OF INVESTIGATOR DATE

2.2 Consent Forms for Adults

INFORMATION AND CONSENT TO PARTICIPATE IN RESEARCH

Exercise and Bone Turnover

You are being invited to participate in a research study being conducted by the investigators listed below. Prior to participating in this study please read this form to find out about the purpose and the tests of this study. For the tests you will have to visit the Exercise Physiology Laboratory (WH 23, Brock University). This study is part of the Faculty of Applied Health Sciences of Brock University.

INVESTIGATORS:	**DEPARTMENT:**	**CONTACT:**
Dr. Bareket Falk	FAHS, Brock University	(905) 688-5550 ex. 4979
Kimberly Kish	FAHS, Brock University	(905) 688-5550 ex. 5623

PURPOSE:

The purpose of the study is to investigate the acute response and recovery of bone metabolism induced by jumping exercise over a period of 24 hours in children and adults.

DESCRIPTION OF TESTING PROCEDURES:

If you agree to volunteer for this study, you will visit our laboratory for one session of testing, lasting approximately **1.5 hours** and another session, 24 hours later, lasting approximately **30 min**. At the end of the study, you will be given a summary of the findings, upon request. It is recommended that you come for the measurements in shorts and a short sleeved shirt.

You will undergo the measurements and procedures listed below; please note that in all questionnaires, you may choose not to answer any question.

C. Pre-exercise Assessments:

5. You will complete several questionnaires, outlining your medical history, physical activities and nutritional habits. Please be aware that the medical history questionnaire includes questions about drug use, alcohol use and smoking. In all questionnaires, you may choose not to answer any question without penalty.

6. Body Composition: we will measure your height, weight and percent body fat. Percent body fat will be estimated using skinfold thicknesses and bioelectrical

impedance analysis (BIA). The BIA assessment requires you to stand on a weight scale and grasp handles. A mild electrical current (50kHz, 800μA) will pass through your hands to your feet. This current cannot be felt and causes no harm. Valid measurements require abstinence from exercise, alcohol consumption, and eating/drinking for at least 12, 24 and 4 hours, respectively, prior to testing.

7. We will determine your bone strength using the Sunlight Omnisense™ ultrasound system. This procedure involves the application of gel to the forearms and the lower legs and moving an ultrasound probe over these regions. This procedure is quick and causes no discomfort.

8. Blood Analysis: A total of 4 blood samples will be collected to determine biochemical markers affecting bone turnover: pre-exercise, immediately after exercise, 60 min and 24 hours post exercise. The blood samples will be drawn using a standard venipuncture technique performed by a certified lab assistant. Up to 10ml will be withdrawn. It should be noted that the venous blood drawing procedure is a routine procedure performed by a certified technician and offers minimal risk to participants. In rare instances, participants may experience slight pain and/or tingling in the area and/or a minor bruise from the needle. However, with the use of anaesthetic creams (e.g., Emla), which we use in the laboratory, any sensation of pain is minimal.

D. Exercise Protocol:

Participants will begin with a warm-up activity that will consist of 5 minutes of low intensity jogging with a series of stretching exercises. The exercise protocol will be designed to provide high impact, weight-bearing loads in the form of circuit training stations. Participants will be instructed to rotate through the five stations, which will be composed of jumping jacks, lunge jumps hopping, jumping over various obstacles (ie, hurdles) and drop jumps, for a total of 3 sets. Each station will take 2 minutes and the participants will then be instructed to progress to the next one. A recovery period of 2 minutes will be given between each set.

CONFIDENTIALITY:

All your data collected during this study will remain confidential and will be stored in offices and on secured computers to which only the principal and co-investigators have access. You should be aware that the results of this study will be made available to scientists, through publication in a scientific journal but your name and any personal data will not appear in compiling or publishing these results. Data will be kept for 5 years after the date of publication, at which time all information will be destroyed. Additionally, you will have access to your own data, as well as group data when it becomes available and

if you are interested. This can be provided to you by simply contacting the principal investigators.

PARTICIPATION AND WITHDRAWL:

You can choose whether to participate in this study or not and may remove your data from the study if you wish. You may also refuse to answer any questions posed to you during the study and still remain as a subject in the study. The investigators reserve the right to withdraw you from the study if they believe that it is necessary.

RISKS AND BENEFITS:

Participation will allow you to become exposed to a research protocol, contribute to the advancement of science and, gain personal and general knowledge about the human body. All results will be provided to you upon request.

The only foreseeable risks involved in participation include:

c) Possible muscle soreness within 48 hours of the test. If this occurs, it will only be temporary.
d) In rare instances, participants may experience slight pain and/or tingling in the area and/or a minor bruise from the venous blood draw.

RIGHTS OF RESEARCH PARTICIPANTS:

You will receive a signed copy of this consent form. You may withdraw your consent to participate in this study at any time, and you may also discontinue participation at any time without penalty. In signing this consent form or in participating in this study you are not waiving any legal claims or remedies. This study has been reviewed and received clearance from the Brock University Research Ethics Board (file # 12-051). If you have any pertinent questions about your rights as a research participant, please contact the Brock University Research Ethics Officer (905 688-5550 ext 3035, reb@brocku.ca).

INFORMATION:

Please contact Dr. Bareket Falk at 905 688-5550 (ext. 4979), bfalk@brocku.ca or Kimberly Kish at 905 688-5550 (ext. 5623), kk11om@brocku.ca if you have any questions about the study.

I HAVE READ AND UNDERSTAND THE ABOVE EXPLANATION OF THE PURPOSE AND PROCEDURES OF THE PROJECT. I HAVE ALSO RECEIVED A SIGNED COPY OF THE INFORMATION AND CONSENT FORM. MY QUESTIONS HAVE BEEN ANSWERED TO MY SATISFACTION AND I AGREE TO PARTICIPATE IN THIS STUDY.

SIGNATURE OF PARTICIPANT DATE

PRINTED NAME OF PARTICIPANT

In my judgment the participant is voluntarily and knowingly giving informed consent and possesses the legal capacity to give informed consent and participate in this research study.

SIGNATURE OF INVESTIGATOR DATE

Appendix 3 Questionnaires

3.1 Subject Screening and Medical History Questionnaire

SUBJECT SCREENING AND MEDICAL HISTORY QUESTIONNAIRE

Name: _____ Date: _____

Date of Birth: _____

Dominant Hand: _____ Dominant Leg: _____

Your responses to this questionnaire are confidential. If you answer "YES" to any of the following questions, please give additional details in the space provided and discuss the matter with one of the investigators. You may refuse to answer any of the following questions.

1. Have you ever had any major joint instability or ongoing chronic pain such as in the knee, back or elbow? **YES** **NO**

2. Are you currently taking any medication (including aspirin) or have you taken any medication in the last two days? **YES** **NO**

3. Have you taken any medication in the past six months? **YES** **NO**

4. Is there any medical condition with which you have been diagnosed and are under the care of a physician (e.g. asthma, diabetes, anorexia)? **YES** **NO**

5. Do you, or have you in the past, consumed any alcohol on a regular basis? **YES** **NO**

6. Do you, or have you in the past, smoked on a regular basis? **YES** **NO**

7. Are you, or have you in the past, engaged in any extreme diet? **YES** **NO**

8. Do you, or have you in the past, consumed any nutritional supplements (e.g. calcium, multi-vitamin) on a regular basis? **YES** **NO**

9. Do you, or have you in the past, engaged in physical activity on a regular basis? **YES** **NO**

10. Have you had any fractures? **YES** **NO**

3.2 Sexual Maturation

Male Pubertal Stage

FACULTY OF APPLIED HEALTH SCIENCES - BROCK UNIVERSITY

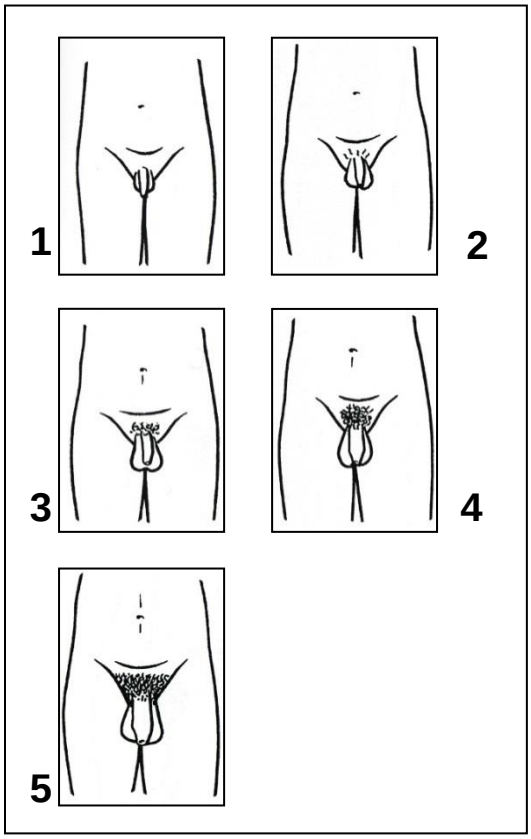
NAME: _____

This scale is to assess your maturational level. For both columns, please choose the appropriate stage and write the corresponding number on this piece of paper.

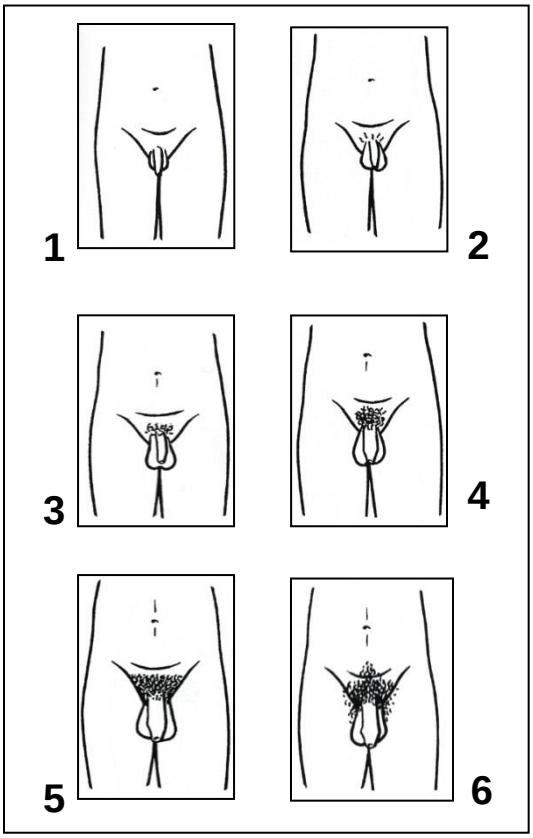
Column #1: _____ (corresponding number)

Column #2: _____ (corresponding number)

 # 1: PLEASE LOOK AT PENIS SIZE ONLY



2: PLEASE LOOK AT PUBIC HAIR ONLY



3.3 Physical Activity

3.3.1 Godin-Shepherd Leisure-Time Exercise Questionnaire

GODIN-SHEPHARD LEISURE-TIME EXERCISE QUESTIONNAIRE

1. Considering a **7-day period** (a week), how many times on the average do you do the following kinds of exercise for **more than 15 minutes** during your **free-time** (write on each line the appropriate number)?

Times PerWeek

(a) STRENUOUS EXERCISE (HEART BEATS RAPIDLY) _____

(i.e. running, jogging, hockey, football, soccer, squash, basketball, cross country skiing, judo, roller skating, vigorous swimming, vigorous long distance bicycling)

(b) MODERATE EXERCISE (NOT EXHAUSTING) _____

(i.e. fast walking, baseball, tennis, easy bicycling, volleyball, badminton, easy swimming, alpine skiing, popular and folk dancing)

(b) MILD EXERCISE (MINIMAL EFFORT) _____

(i.e. yoga, archery, fishing from river bank, bowling, horseshoes, golf, snow-mobiling, easy walking)

2. Considering a **7-day period** (a week), during your leisure-time, how often do you engage in any regular activity long enough to work up a sweat (heart beats rapidly)?

1. OFTEN	**2. SOMETIMES**	**3. NEVER/RARELY**
☐	☐	☐

3.3.2 Past Year Physical Activity Questionnaire

PHYSICAL ACTIVITY AND EXERCISE

NAME _____ **DATE** _____
ID _____ **CLASS** _____ **GRADE** _____

1. How many of the past 14 days have you done at least 20 minutes od exercise **hard** enough to make you breath heavily and make your heart beat fast? (Hard exercise includes, for example, playing basketball, jogging, fast dancing or bicycling; include time in physical education class)

   1. None
   2. 1 to 2 days
   3. 3 to 5 days
   4. 6 to 8 days
   5. 9 or more days

2. How many of the past 14 days have you done at least 20 minutes of **light** exercise that **was not** hard enough to make you breath heavily and make your heart beat fast? (Light exercise includes, for example, playing baseball, walking or slow bicycling; include time in physical education class)

   1. None
   2. 1 to 2 days
   3. 3 to 5 days
   4. 6 to 8 days
   5. 9 or more days

4. During a normal week, how many hours **a day** do you watch television and videos, or play computer or video games before and after school?

   1. None
   2. 1 hour or less
   3. 2 to 3 hours
   4. 4 to 5 hours
   5. 6 or more hours

5. During the last 12 months, how many team or individual sports or activities did you participate in on a **competitive** level, such as varsity or junior varsity sports, intramurals, YMCA or other out-of-school programs.

   1. None
   2. 1 activity
   3. 2 activities
   4. 3 activities
   5. 4 or more activities

What activities did you compete in?

1. _____
2. _____
3. _____
4. _____
5. _____
6. _____
7. _____

PAST YEAR LEISURE-TIME PHYSICAL ACTIVITY

Check all activities that you did <u>at least ten times</u> in the **PAST YEAR**. Do not include time spent in school physical education classes. Make sure you include all sport teams that you participated in during the last year.

____ Aerobics
____ Band/Drill Team
____ Baseball
____ Basketball
____ Bicycling
____ Bowling
____ Cheerleading
____ Dance Class
____ Football
____ Garden/Yard Work
____ Gymnastics
____ Hiking
____ Ice Skating
____ Roller Skating
____ Running for Exercise
____ Skateboarding
____ Snow Skiing
____ Soccer
____ Softball
____ Street Hockey
____ Swimming (Laps)
____ Tennis
____ Volleyball
____ Water Skiing
____ Weight Training
____ Wrestling (Competitive)

<u>Others</u>

_____
_____
_____

List each activity that you checked above in the "Activity" box below, check the months you did each activity and then estimate the amount of time spent in each activity.

Activity	Jan	Feb	Mar	Apr	May	Jun	Jly	Aug	Sep	Oct	Nov	Dec	Months per Year	Days Per Week	Minutes Per Day

LEISURE-TIME PHYSICAL ACTIVITY CALCULATIONS

1. For each activity:

$$\frac{(\text{\# months/year}) \times (4.3\ \text{weeks/month}) \times (\text{\# days/week}) \times (\text{\# minutes/day})}{(60\ \text{minutes/hour}) \times (52\ \text{weeks/year})} = \text{hours/week of activity}$$

2. Sum the hours/week for each activity to determine the total physical activity estimate for the past year.

3. To express the results in MET-hours/week, multiply the hours/week for each activity (derived in step 1) by the activity's MET equivalent (obtained from existing charts).

Example: Basketball (MET equivalent = 9)

$$\frac{(4\ \text{months/year}) \times (4.3\ \text{weeks/month}) \times (4\ \text{days/week}) \times (60\ \text{minutes/day})}{(60\ \text{minutes/hour}) \times (52\ \text{weeks/year})} = 1.3\ \text{hours/week}$$

(9 METS) x (1.3 hours/week) = 11.9 MET-hours/week, or 11.9 $kcal/kg^{-1}/wk^{-1}$

Bone-Specific Physical Activity Questionnaire (BPAQ)

SUBJECT ID:	DATE:

1. Please list any sports or other physical activities you have participated in regularly. Please tick the boxes to indicate how old you were for each sport/activity and how many years you participated for.

Activities \ Age:	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30

Activities \ Age:	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60

Activities \ Age:	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90

Bone-Specific Physical Activity Questionnaire (BPAQ)

SUBJECT ID:	DATE:

2. **Please list the sports or other physical activities (be as specific as possible) you participated in regularly during the last 12 months and indicate the average frequency (sessions per week).**

Activity: _____ Frequency (per week): _____

Activity: _____ Frequency (per week): _____

Activity: _____ Frequency (per week): _____

Activity: _____ Frequency (per week): _____

Activity: _____ Frequency (per week): _____

Activity: _____ Frequency (per week): _____

Activity: _____ Frequency (per week): _____

Activity: _____ Frequency (per week): _____

3.4 24-hour Nutritional Recall Questionnaire

24 HOUR NUTRITIONAL RECALL

Name: _____ Date: _____

24 Hour Recall Date: _____

Nutritional Intake:

_____

_____

_____

_____

_____

_____

_____

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Appendix 4 Data Collection Sheets

EXERCISE AND BONE TURNOVER **ID: _____**

SECTION 1: PARTICIPANT INFORMATION		
Participant ID #: _____	First and Last Name: _____	Date of Birth: _____ / _____ / _____ (month) (day) (year)
Visit Date: _____	Age: _____	Dominant Limbs: _____ _____ (Arm) (Leg)

SECTION 2: CONSENTS, QUESTIONNAIRES AND DATA COLLECTION
1.Consent form: _____
2. Subject Screening and Medical History Questionnaire: _____
3. Anthropometric Measurements: _____
4. 24 Hour Nutrition Recall: _____
5. Godin-Shephard Leisure-Time Exercise Questionnaire: _____
6. Past Year Physical Activity Questionnaire: _____
7. Bone-Specific Physical Activity Questionnaire: _____
8. Tanner Staging: _____
9. Bone QUS: _____
10. Blood Sample #1 – Pre-Exercise: _____
11. Plyometric Exercise: _____
12. Accelerometer Worn: _____
13. Blood Sample #2 – 5min Post-Exericse: _____
14. Blood Sample #3 – 1 hour Post-Exercise: _____
15. Blood Sample #4 – 24 hour Post-Exercise: _____
16. Incentive Given: _____

SECTION 3: COMMENTS

EXERCISE AND BONE TURNOVER **ID: _____**

NAME: _____ **DATE: _____**

DOB: _____ **AGE: _____**

DOMINANT ARM: _____ **DOMINANT LEG: _____**

WEIGHT: _____ **HEIGHT: _____** **BMI: _____** **BF%: _____**

ARM AND LEG LENGTH (CM):

	TRIAL 1	TRIAL 2	TRIAL 3	TRIAL 4 (>1cm difference)	MEDIAN
UPPER ARM					
UPPER LEG					

SKINFOLD THICKNESS (MM):

	TRIAL 1	TRIAL 2	TRIAL 3	TRIAL 4 (>1mm difference)	MEDIAN
TRICEPS					
SUBSCAPULA					
ANTERIOR THIGH					

EXERCISE AND BONE TURNOVER **ID:** _____

Bone Strength Data:

Sunlight Omniscence™

DOMINANT:

Side (circle)	Site	SOS	T-Score	Z-Score
L or R	Dominant Radius			
L or R	Dominant Tibia			

NON-DOMINANT:

Side (circle)	Site	SOS	T-Score	Z-Score
L or R	Non-Dominant Radius			
L or R	Non-Dominant Tibia			

WARM-UP PROTOCOL:

TIME (minutes)	WATTS	HEART RATE (bpm)
1:00		
2:00		
3:00		
4:00		
5:00		

EXERCISE AND BONE TURNOVER **ID:** _____

ONSET OF EXERCISE: _____ **COMPLETION OF EXERCISE:** _____

SET #1			
	START TIME	**END TIME**	**HEART RATE**
DROP JUMPS			
LUNGE JUMPS			
HURDLES			
1 FOOT HOPS			
JUMPING JACKS			
FINAL HEART RATE			
SET #2			
	START TIME	**END TIME**	**HEART RATE**
DROP JUMPS			
LUNGE JUMPS			
HURDLES			
1 FOOT HOPS			
JUMPING JACKS			
FINAL HEART RATE			
SET #3			
	START TIME	**END TIME**	**HEART RATE**
DROP JUMPS			
LUNGE JUMPS			
HURDLES			
1 FOOT HOPS			
JUMPING JACKS			
FINAL HEART RATE			

EXERCISE AND BONE TURNOVER **ID:** _____

Blood Sample Timing:

	TIME OF SAMPLE	TIME TO CENTRIFUGE
SAMPLE #1 – PRE-EXERCISE		
SAMPLE #2 – 5MIN POST-EXERCISE		
SAMPLE #3 – 1HR POST-EXERCISE		
SAMPLE #4 – 24HR POST-EXERCISE		

COMMENTS:

Appendix 5 Additional Data, not appearing in main body of thesis

Table 12. Participant SOS (m/s) and Z-scores from the QUS measurement for the non-dominant limbs for men and boys.

	Adults (n=18)	Children (n=12)	p-value
Non-Dominant Radius SOS	4153.6 ± 115.8	3794.5 ± 116.1	<0.001*
Non-Dominant Radius Z-Score	0.8 ± 1.0	0.1 ± 1.2	NS
Non-Dominant Tibia SOS	3963.1 ± 85	3706.3 ± 72.4	<0.001*
Non-Dominant Tibia Z-Score	-0.1 ± 0.8	0.5 ± 0.7	<0.05*

All values are expressed as means ± SD. Significance was set at $p<0.05$ difference between groups.
SOS – speed of sound

Table 13. Time spent in moderate, vigorous or very vigorous activity (minutes), the counts in the vertical and horizontal axis and the total time for the exercise protocol

	Adults (n=18)	Children (n=12)	p-value
Moderate Activity	3.3 ± 1.3	5.0 ± 1.4	<0.002*
Vigorous Activity	1.5 ± 0.5	1.9 ± 0.5	NS
Very Vigorous Activity	6.4 ± 2.2	4.6 ± 0.6	<0.007*
Vertical Axis Count	140519 ± 46266	82840 ± 12751	<0.001*
Horizontal Axis Count	65849 ± 20202	49911 ± 10488	<0.02*
Total Exercise Time	27.5 ± 6.1	21.4 ± 3.4	<0.004*

All values are expressed as means ± SD. Significance was set at $p<0.05$ difference between groups.

Table 11. Heart rates (bpm) after each exercise throughout each set

	Adults (n=18)	Children (n=12)
#1 DJ	165 ± 10.6	170 ± 16.2
#1 LJ	170 ± 13.8	178 ± 14.2
#1 HJ	167 ± 14.8	173 ± 11.0
#1 S-L Hop	178 ± 10.4	183 ± 10.5
#1 JJ	174 ± 13.1	180 ± 14.5
#2 DJ	164 ± 11.4	172 ± 10.3
#2 LJ	172 ± 11.5	178 ± 14.3
#2 HJ	169 ± 13.6	174 ± 13.9
#2 S-L Hop	178 ± 10.5	185 ± 10.7
#2 JJ	176 ± 9.8	182 ± 9.5
#3 DJ	166 ± 11.0	173 ± 13.2
#3 LJ	172 ± 11.5	180 ± 16.0
#3 HJ	173 ± 13.4	174 ± 16.5
#3 S-L Hop	181 ± 11.4	185 ± 10.5
#3 JJ	177 ± 9.6	183 ± 12.9

All values are expressed as means ± SD. Significance was set at $p<0.05$ difference between groups.
DJ – drop jump, LJ – lunge jump, HJ – hurdle jump, S-L – single-leg, JJ – jumping jacks

Raw Data - Men

ID	date of birth	test date	age in days	age in years	group	ethnicity	fractures	family history - osteoporosis	extreme diet	smoking	alcohol	supplements - Ca	supplements - vit D	medication	med details	medical condition
001	07/08/90	11/07/12	8158	22.35	2	1	1	0	0	0	1	0	0	0		
002	02/28/89	11/12/12	8658	23.7	2	1	0	0	0	0	1	0	0	0		
003	08/19/89	11/21/12	8495	23.3	2	1	0	0	0	0	0	0	0	0		
004	07/02/84	11/27/12	10375	28.4	2	1	0	0	0	0	0	0	1	0		
005	11/17/90	11/29/12	8048	22.0	2	1	0	0	0	0	0	0	0	0		
006	08/01/93	11/29/12	7060	19.3	2	1	0	0	0	0	0	0	0	1	Ventolin & Flovent	2
007	01/23/89	12/03/12	8715	23.9	2	1	0	0	0	0	0	0	0	1	Thyroxine	
009	06/15/93	12/06/12	7114	19.5	2	1	1	0	0	0	0	0	0	0		
0010	12/11/93	01/22/13	6982	19.1	2	1	1	0	0	0	0	0	0	0		
0011	05/23/88	01/28/13	9016	24.7	2	1	0		0	0	0	0	0	0		
0012	03/27/89	02/04/13	8715	23.9	2	1	1	0	0	0	1	0	0	0		
0013	06/02/93	02/07/13	7190	19.7	2	1	1	1	0	0	0	0	0	0		
0014	01/15/91	02/11/13	8063	22.1	2	1	1		0	0	1	0	1	0		
0015	06/17/94	02/27/13	6830	18.7	2	1	0	0	0	0	0	0	0	1		
0016	11/25/93	02/27/13	7034	19.3	2	1	1	0	0	1	0	0	0	0		
0017	02/16/88	03/07/13	9151	25.1	2	1	1		0	0	0	0	0	1	Amoxacillan, cyprofloxacin	0
0018	03/18/91	03/18/13	8036	22.0	2	1	1		0	0	0	0	0	1	Clonazepam	0
0019	05/19/85	04/13/13	10191	27.9	2	1	1		0	0	0	0	0	0		

ID	wt (kg)	ht (cm)	ht (m)	BMI	triceps	subscap	2 skin folds	body fat % - SF	Fat Free Mass	Body Fat % - BIA	Sitting_Height (cm)	Seated_Height (minus table)	Leg_length (cm)	Yrs_PHV	Tanner - P	Tanner - PH
001	82.30	182.30	1.82	24.85	9.10	11.30	20.40	15.85	69.26	15.10						
002	106.3	191.10	1.91	29.11	22.30	25.20	47.50	33.93	70.23	27.90						
003	71.25	169.30	1.69	24.86	4.40	7.30	11.70	7.56	65.86	9.90						
004	89.10	192.60	1.93	24.02	7.00	10.00	17.00	12.76	77.73	9.8						
005	67.50	173.10	1.73	22.53	6.40	6.80	13.20	9.08	61.37	9.40						
006	86.90	186.50	1.87	24.98	9.80	10.20	20.00	15.50	73.43	9.40						
007	75.90	175.30	1.75	24.70	6.80	8.00	14.80	10.66	67.81	11.30						
009	71.40	175.40	1.75	23.21	6.90	7.80	14.70	10.56	63.86	11.40						
0010	60.70	173.10	1.73	20.26	13.60	10.60	24.20	19.10	49.11	14.60						
0011	87.80	183.20	1.83	26.16	8.00	9.00	17.00	12.76	76.60	12.50						
0012	70.1	180.00	1.800	21.64	10.20	10.40	20.60	16.031	58.88	21.60						
0013	82.40	180.10	1.80	25.40	7.00	9.20	16.20	12.002	72.51	16.20						
0014	75.30	176.80	1.77	24.09	9.20	9.80	19.00	14.602	64.31	14.80						
0015	67.60	173.00	1.73	22.59	11.40	10.40	21.80	17.076	56.04	14.90						
0016	88.70	188.50	1.89	24.96	9.40	9.80	19.20	14.783	75.57	15.80						
0017	73.30	180.50	1.81	22.50	8.80	9.20	18.00	13.688	63.26	14.40						
0018	69.20	178.90	1.79	21.62	10.80	13.20	24.00	18.932	56.12	12.30						
0019	88.40	182.70	1.83	26.48	17.40	14.50	31.90	24.958	66.30	22.90						

ID	stren ex	mod ex	mild ex	activity score	PYPA - Hard Exercise	PYPA - Light Exercise	Hours spent watching TV	# of competitive activities	Activity 1	Activity 2	Activity 3	Activity 4	PYPA - Act 1	PYPA - Act 1 - h/wk	PYPA - Act 1 MET eqvt	PYPA - Act 1 - METhr/wk
001	3	2	4	49	3	4	5	5	4	2	3		1	0.740	6.0	4.440
002	8	8	8	128	4	3	3	1					6	1.323	5.0	6.615
003	14	7	7	182	5	5	3	3	3	14			11	1.985	7.3	14.488
004	5	5	7	91	4	5	3	4	15	2	23		15	2.977	9.8	29.174
005	5	6	7	96	5	5	2	2	24				17	0.496	6.5	3.225
006	5	1	4	62	4	4	3	5	2	4	3		7	0.207	7.5	1.550
007	3	15	7	123	5	3	1	2	19				19	19.846	12.5	248.077
009	7	1	3	77	3	2	4	3	20	3			11	0.496	7.3	3.622
0010	2	1	1	26	3	3	3	2	3				6	0.413	5.0	2.067
0011	2	5	6	61									17	3.969	6.5	25.800
0012	2	2	7	49	3	3	3	1					11	0.620	7.3	4.527
0013	5	3	7	81	4	3	5	2	23				20	0.992	8.0	7.938
0014	3	4	4	59									17	0.413	6.5	2.687
0015	4	2	6	64	2	3	4	3	2	17			17	0.496	6.5	3.225
0016	4	4	4	68	4	5	3	3	2	4			17	0.165	6.5	1.075
0017	5	5	7	91	2	5	3	4	21	5	7		7	1.488	7.5	11.163
0018	3	4	7	68	3	5	3	2	19				7	5.292	7.5	39.692
0019	2	2	5	43	3	4	4	3	3	21			3	1.985	8.0	15.877

ID	PYPA - Act 2	PYPA - Act 2 - h/wk	PYPA - Act 2 MET eqvt	PYPA - Act 2 - METhr/wk	PYPA - Act 3	PYPA - Act 3- h/wk	PYPA - Act 3 MET eqvt	PYPA - Act 3 - METhr/wk	PYPA - Act 4	PYPA - Act 4 - h/wk	PYPA - Act 4 MET eqvt	PYPA - Act 4 - METhr/wk	PYPA - Act 5	PYPA - Act 5 - h/wk	PYPA - Act 5 MET eqvt	PYPA - Act 5 - METhr/wk
001	2	0.660	10.0	6.620	3	0.280	8.0	2.205	4	0.124	4.0	0.496	5.0	0.248	11.8	2.927
002	7	3.184	7.5	23.877	8	3.473	4.0	13.892	9	7.442	6.0	44.652	10.0	1.158	3.5	4.052
003	7	1.240	7.5	9.303	12	2.315	3.8	8.798	9	0.662	6.0	3.969	13.0	1.158	7.0	8.104
004	16	1.819	5.0	9.096												
005	7	0.827	7.5	6.202	5	2.481	11.8	29.273	16	0.992	5.0	4.962				
006	8	1.488	4.0	5.954	3	0.930	8.0	7.442	5	0.248	11.8	2.927	18.0	1.654	7.0	11.577
007	7	7.938	7.5	59.538	16	3.473	5.0	17.365								
009	17	0.662	6.5	4.300	20	0.413	8.0	3.308	3	2.977	8.0	23.815	5.0	0.827	11.8	9.758
0010	8	0.413	4.0	1.654	9	0.992	6.0	5.954	13	0.248	7.0	1.737	5.0	0.496	11.8	5.855
0011	7	1.654	7.5	12.404	5	1.654	11.8	19.515	18	0.620	7.0	4.341	16.0	5.954	5.0	29.769
0012	17	0.413	6.5	2.687	5	0.248	11.8	2.927	18	0.310	7.0	2.171	1.0	0.145	6.0	0.868
0013	8	4.962	4.0	19.846	5	2.481	11.8	29.273	21	0.662	7.8	5.160	16.0	7.442	5.0	37.212
0014	7	1.985	7.5	14.885	8	0.744	4.0	2.978	9	3.969	6.0	23.815	5.0	2.233	11.8	26.346
0015	8	0.207	4.0	0.827	9	0.248	6.0	1.488	13	0.248	7.0	1.737	5.0	0.868	11.8	10.246
0016	7	0.248	7.5	1.861	8	0.331	4.0	1.323	5	0.551	11.8	6.505	18.0	1.985	7.0	13.892
0017	9	0.496	6.0	2.977	13	0.248	7.0	1.737	5	0.827	11.8	9.758	30.0	0.413	5.3	2.191
0018	5	2.646	11.8	31.225	9	2.315	6.0	13.892	15	4.465	9.8	43.761				
0019	21	0.331	7.8	2.580												

ID	PYPA - Act 6	PYPA - Act 6 - h/wk	PYPA - Act 6 MET eqvt	PYPA - Act 6 - METhr/wk	PYPA - Act 7	PYPA - Act 7 - h/wk	PYPA - Act 7 MET eqvt	PYPA - Act 7 - METhr/wk	PYPA - Act 8	PYPA - Act 8 - h/wk	PYPA - Act 8 MET eqvt	PYPA - Act 8 - METhr/wk	PYPA - Act 9	PYPA - Act 9 - h/wk	PYPA - Act 9 MET eqvt	PYPA - Act 9 - METhr/wk
001																
002																
003	5.0	1.9	11.8	23.418	3.0	0.554	8.0	4.431	14.0	2.977	6.0	17.862				
004																
005																
006	1.0	0.3	6.0	1.675	4.0	0.372	3.0	1.116	16.0	0.992	5.0	4.962				
007																
009	18.0	0.3	7.0	1.737	21.0	0.372	7.8	2.902	1.0	1.240	6.0	7.442	22.0	1.323	7.3	9.658
0010	22.0	0.3	7.3	2.415	10.0	0.992	3.5	3.473	3.0	0.744	8.0	5.954				
0011																
0012	4.0	0.1	3.0	0.372	10.0	3.473	3.5	12.156								
0013	3.0	0.7	8.0	5.292												
0014	18.0	0.3	7.0	1.929	21.0	0.413	7.8	3.225	4.0	0.662	3.0	1.985	14.0	4.465	6.0	26.792
0015	18.0	3.3	7.0	23.154	1.0	0.186	6.0	1.116	10.0	0.868	3.5	3.039				
0016	1.0	0.2	6.0	0.992	4.0	1.034	3.0	3.101	16.0	7.277	5.0	36.385				
0017	21.0	1.5	7.8	11.610	16.0	1.985	5.0	9.923								
0018																
0019																

ID	PYPA - Act 10	PYPA - Act 10 - h/wk	PYPA - Act 10 MET eqvt	PYPA - Act 10 - METhr/wk	PYPA - Act 11	PYPA - Act 11 - h/wk	PYPA - Act 11 MET eqvt	PYPA - Act 11 - METhr/wk	Total MET hrs/week	BPAQ - Current	BPAQ - Past	BPAQ - Total	Current Impact Exercise	Current Impact Exercise Frequency
001									13.761	9.026	188.57	98.797	1	8
002									89.036	0.537	47.128	23.833	1	1
003									77.838	19.193	65.313	42.253	1	8
004									38.270	0.485	17.657	9.071	0	0
005									43.661	7.165	91.451	49.308	1	6
006									24.510	13.533	123.08	68.308	1	10
007									324.981	0.497	51.12	25.809	1	2
009	16.0	13.892	5.0	69.462					53.882	7.891	63.204	35.547	1	12
0010									19.780	12.451	96.01	54.231	1	4
0011									62.061	8.556	91.566	50.061	1	8
0012									12.685	9.41	88.506	48.958	1	6
0013									67.510	9.004	151.03	80.019	1	12
0014									75.072	17.09	75.509	46.299	1	8
0015									33.470	11.101	56.084	33.593	1	7
0016									48.141	20.132	38.735	29.434	1	4
0017									37.245	4.953	42.224	23.589	1	9
0018									128.570	3.819	215.27	109.55	1	3
0019									18.457	5.756	33.527	19.642	1	3

ID	Accelerometer - Sedentary	Accelerometer - Light	Accelerometer - Lifestyle	Accelerometer - Moderate	Accelerometer - Vigorous	Accelerometer - Very Vigorous	Accelerometer - Axis 1 Counts	Accelerometer - Axis 2 Counts	Cylce Ergometer WU Min#1 - HR	Cylce Ergometer WU Min#2 - HR	Cylce Ergometer WU Min#3 - HR	Cylce Ergometer WU Min#4 - HR	Cylce Ergometer WU Min#5 - HR
001	5.92	3.07	2.17	2.28	1.13	8.43	180079	69621					
002	18.58	4.95	4.32	4.70	2.32	8.13	167383	71823	147	144	166	170	174
003	10.68	2.80	2.12	4.17	1.08	8.15	173157	83602	139	147	151	163	169
004	6.97	3.27	3.22	3.13	1.23	5.18	119752	49292			126	136	148
005	17.65	6.07	5.33	7.30	2.32	13.33	292909	132894	131	147	159	167	173
006	8.13	2.27	1.68	2.37	1.10	5.45	123059	54322	115	126	133	141	147
007	4.20	1.00	1.57	2.83	1.13	5.27	126232	49837					
009	9.03	3.17	3.47	4.75	2.42	7.17	154942	72517	127	139	149	151	161
0010	14.65	1.90	2.02	2.53	1.37	5.53	112480	43579	128	149	162	174	182
0011	62.48	3.82	2.05	3.52	1.32	7.05	157668	55579	116	124	124	136	144
0012	15.13	4.47	2.13	1.95	0.90	5.42	126841	50794	133	157	169	176	181
0013	9.08	2.55	2.48	2.68	1.37	5.28	116890	56381	136	155	164	171	177
0014	6.13	4.68	3.02	3.65	1.93	6.60	151494	78653	113	132	139	146	154
0015	17.50	2.07	1.78	3.60	1.40	5.15	119293	76601	123	140	160	181	193
0016	16.55	3.05	2.60	3.45	1.57	4.78	101549	59832	132	144	150	162	174
0017	20.18	2.18	1.22	1.40	1.73	5.60	110064	58593	138	150	159	165	175
0018	10.12	1.47	1.78	2.68	1.80	5.13	107034	64600	126	147	156	170	175
0019	16.00	2.80	2.37	2.85	1.03	3.95	88530	56762	112	129	141	147	160

ID	Onset of Exercise Time	Completion of Exercise Time	Total Time (minutes)	Recovery HR	Set #1 - Drop Jumps HR	Set #1 - Lunge Jumps HR	Set #1 - Hurdle Jumps HR	Set #1 - Single-leg Hops HR	Set #1 - Jumping Jacks HR	Recovery HR
001										
002	9:14 AM	9:56 AM	42		186	196	185	195	186	150
003	9:19 AM	9:47 AM	28		160	167	160	183	180	123
004	9:25 AM	9:47 AM	22	93	156	165	141	168	167	
005	8:54 AM	9:18 AM	24		160	155	161	174	172	95
006	9:31 AM	9:51 AM	20	120	148	164	151	169	169	106
007	9:12 AM	9:27 AM	15	143	163	164	161	178	176	110
009	9:56 AM	10:25 AM	31	119	161	174	166	176	163	131
0010	10:39 AM	11:06 AM	27	124	160	153	146	159	141	106
0011	9:40 AM	10:11 AM	31	106	162	144	150	168	162	90
0012	9:37 AM	10:06 AM	29	103	169	179	176	179	172	111
0013	9:31 AM	9:54 AM	23	140	181	180	185	190	190	115
0014	9:25 AM	9:52 AM	27		164	164	170	176	173	118
0015	9:28 AM	9:59 AM	31		186	189	191	197	197	140
0016	9:36 AM	10:07 AM	31		162	180	180	180	186	108
0017	9:21 AM	9:55 AM	34	131	175	184	181	188	181	107
0018	9:33 AM	9:57 AM	24	134	162	172	170	179	180	129
0019	9:52 AM	10:21 AM	29	110	156	156	161	166	164	100

ID	Set #2 - Drop Jumps HR	Set #2 - Lunge Jumps HR	Set #2 - Hurdle Jumps HR	Set #2 - Single-leg Hops HR	Set #2 - Jumping Jacks HR	Recovery HR	Set #3 - Drop Jumps HR	Set #3 - Lunge Jumps HR	Set #3 - Hurdle Jumps HR	Set #3 - Single-leg Hops HR	Set #3 - Jumping Jacks HR
001											
002	186	192	183	191	188	142	186	188	198	202	188
003	157	164	160	178	175	124	160	169	159	175	159
004	165	170	155	170	169	130	168	175	159	175	168
005	151	157	149	175	174	115	156	159	166	171	173
006	161	174	163	168	168	100	167	176	165	174	174
007	170	178	180	187	186	109	170	186	188	188	189
009	166	173	168	164	167	113	168	169	174	180	179
0010	142	144	137	161	155	105	144	148	143	167	167
0011	155	168	174	180	174	107	168	168	174	192	186
0012	161	173	171	176	170	121	166	169	166	163	168
0013	179	185	187	194	184	106	186	189	189	196	188
0014	161	169	169	176	176	120	162	173	175	180	175
0015	176	184	185	192	194	126	178	185	186	193	192
0016	174	180	180	186	186	108	168	174	180	186	180
0017	173	179	182	190	178	105	171	179	173	185	178
0018	160	173	167	180	179	107	157	170	176	177	177
0019	151	160	166	165	165	93	151	154	163	164	165

ID	dom arm	dom leg	D radius	DR z score	Dt tibia	DT z score	ND radius	NDR z score	NDt tibia	NDT z score	Total kCal Intake (kcal)	Total kCal Intake per kg (kcal/kg)
001	L	L	4238	1	4097	1	4223	1	4050	0	2296	28
002	R	R	4110	0	4034	0	4158	1	4032	0	1766	17
003	R	R	4324	2	3969	0	4237	1	3954	0	2636	37
004	R	R	4321	2	4127	1	4405	3	4085	1	2939	33
005	R	R	4126	1	4115	1	4084	0	4106	1	1955	29
006	R	R	4042	0	3891	-1	4022	0	3890	-1	2370	27
007	L	R	4014	-1	3999	0	4118	0	4021	0	2689	35
009	R	R	4002	0	3908	-1	3974	-1	3952	0	4831	68
0010	R	R	4047	1	3989	1	4164	2	3947	0	3723	61
0011	L	R	3919	-1	3981	0	4011	-1	3864	-1	1613	18
0012	R	R	4052	0	4124	1	4156	1	4077	1	2203	31
0013	L	R	4140	1	3953	0	4176	1	3969	0	1754	21
0014	R	R	4068	0	3923	-1	4346	2	3896	-1	3367	45
0015	R	R	4022	1	3919	0	4044	1	3915	0	3118	46
0016	R	R	4039	0	3927	0	4168	2	3998	1	3187	36
0017	R	R	4159	1	3935	-1	4030	0	3858	-1	4583	63
0018	R	R	4075	0	3844	-1	4193	1	3821	-2	3451	50
0019	R	R	4076	0	3907	-1	4255	1	3900	-1	1461	17

ID	Total Energy - CHO (g)	Total Energy - FAT (g)	Total Energy - PROTEIN (g)	Total Calcium 24Hr (mg)	Total Vitamin D (ug)	Total Vitamin D (IU)	Total Sodium (mg)	Total Potassium (mg)	Total Phosphorus (mg)	Total Magnesium (mg)	Total Zinc (mg)	Total Caffeine (mg)
001	319	83	79	1069	3	102	3493	2170	1450	277	5	0
002	245	45	103	263	1	66	1084	2749	960	245	13	0
003	284	92	173	1215	6	239	3905	3559	1561	359	10	120
004	281	136	167	1409	13	431	3146	5439	2885	787	23	0
005	368	37	64	776	3	120	1541	4521	1201	546	9	1
006	387	43	126	1580	2	94	2105	5185	1958	768	14	0
007	395	79	123	875	4	116	875	4587	1729	597	12	107
009	602	230	140	1040	3	125	5330	8057	1338	466	4	17
0010	521	124	136	1587	8	257	8955	3730	1598	388	10	118
0011	193	49	111	681	5	229	2296	3706	1524	363	20	0
0012	263	97	91	576	2	83	2894	1936	945	279	8	0
0013	153	59	154	1716	9	357	2512	3027	2119	393	12	284
0014	489	118	125	1446	7	268	3018	5352	2521	881	20	0
0015	318	143	131	871	3	65	5096	800	1397	174	6	0
0016	430	99	144	2455	9	363	7376	4062	2930	377	19	0
0017	544	165	242	1657	11	427	6470	3165	2190	351	22	1
0018	357	147	179	2273	19	811	3822	5919	2924	726	29	15
0019	221	45	60	339	0	8	1095	1878	819	228	5	169

ID	NTX - T1 (nM)	Dilution Factor 1	NTX - T2 (nM)	Dilution Factor 2	NTX - T3 (nM)	Dilution Factor 3	NTX - T4 (nM)	Dilution Factor 4	BAP - T1 (µg/L)	Dilution Factor 1	BAP - T2 (µg/L)	Dilution Factor 2
001	19.61		24.24		21.31		21.22		19.98		18.24	
002	17.48		17.55		16.51		18.87		14.39		15.45	
003	26.93		20.65		20.67		20.67		32.21		35.03	
004	19.05		11.68		13.06		13.40		28.32		27.59	
005	20.20		17.07		13.23		16.66		51.71		56.95	
006	20.84		15.96		17.61		21.17		19.25		20.02	
007	21.21		17.78		17.80		17.78		25.43		23.65	
009	31.73		27.89		22.91		23.79		28.54		29.11	
0010	16.74		14.37		24.42		11.86		29.61		30.77	
0011	21.34		23.66		14.62		21.60		33.31		36.93	
0012	15.06		20.72		10.45		23.50		22.32		24.66	
0013	18.15		24.35		13.40		13.49		28.70		31.30	
0014	25.96		34.22		25.66		21.67		38.43		34.23	
0015	24.28		37.81		33.51		26.75		39.53		40.07	
0016	30.77		30.92		23.08		30.61		57.07		52.44	
0017	28.94		28.65		25.93		16.27		43.92		49.36	
0018	20.42		23.68		21.15		16.39		26.89		29.69	
0019	12.49		16.73		12.03		13.14		26.32		25.37	

ID	BAP - T3 (µg/L)	Dilution Factor 3	BAP - T4 (µg/L)	Dilution Factor 4	OPG - T1 (pmol/L)	OPG - T2 (pmol/L)	OPG - T3 (pmol/L)	OPG - T4 (pmol/L)
001	20.99		19.82		2.46	2.76	2.68	2.21
002	15.55		19.95		1.97	2.47	2.71	2.50
003	39.19		34.09		4.24	3.47	4.38	4.81
004	28.88		27.78		4.19	4.62	4.17	5.35
005	59.01		50.34		5.35	5.99	5.08	4.09
006	17.39		18.49		3.98	4.19	3.91	4.11
007	29.51		24.80		3.18	3.10	3.25	2.39
009	28.71		26.09		3.74	4.16	3.80	3.36
0010	30.77		32.89		4.11	4.24	4.54	4.03
0011	38.94		35.74		1.96	3.02	2.84	2.47
0012	27.34		28.70		2.68	3.19	3.74	3.46
0013	30.76		34.96		3.79	3.78	4.89	4.58
0014	39.99		41.85		4.82	4.96	5.70	6.96
0015	42.61		45.46		4.67	4.41	5.16	8.20
0016	56.23		55.21		2.66	2.97	2.92	2.81
0017	45.69		42.75		2.98	3.75	1.29	2.83
0018	32.23		33.29		2.11	1.95	2.21	2.14
0019	25.97		26.98		2.82	3.75	3.67	3.38

ID	RANKL - T1 (pmol/L)	Dilution Factor 1	RANKL - T2 (pmol/L)	Dilution Factor 2	RANKL - T3 (pmol/L)	Dilution Factor 3	RANKL - T4 (pmol/L)	Dilution Factor 4
001	2.76	276.37	2.28	227.84	2.16	215.89	2.01	201.01
002	1.44	144.38	2.00	200.31	2.68	268.18	1.71	170.78
003	1.19	118.70	0.88	88.41	0.96	96.39	1.03	103.00
004	4.64	463.59	7.19	719.30	2.83	283.39	3.38	337.57
005	3.38	338.23	3.83	382.65	3.33	332.84	3.77	377.44
006	3.68	368.00	4.73	472.98	4.83	482.87	4.64	464.03
007	2.89	288.90	3.35	335.00	3.53	353.19	3.39	339.30
009	3.75	375.34	4.00	400.27	3.63	362.73	3.69	369.05
0010	3.35	335.00	3.35	335.00	3.20	319.83	3.29	328.52
0011	5.56	555.91	4.02	402.33	3.21	320.92	3.48	347.87
0012	1.66	165.95	1.77	177.37	1.35	134.93	2.15	214.55
0013	0.82	82.45	0.97	97.29	0.65	65.42	0.91	90.75
0014	7.91	321.63	9.65	346.83	9.00	280.77	8.11	305.51
0015	5.45	544.62	6.07	607.47	5.17	517.49	4.79	478.56
0016	0.89	89.10	1.53	152.85	1.21	121.12	1.27	127.30
0017	4.72	472.33	3.86	386.38	4.11	410.59	4.39	438.61
0018	4.95	495.07	5.15	515.47	3.99	398.55	5.22	521.54
0019	3.54	353.68	4.20	420.36	3.38	337.54	2.84	283.78

Raw Data - Boys

ID	date of birth	test date	age in days	age in years	group	ethnicity	fractures	family history - osteoporosis	extreme diet	smoking	alcohol	supplements - Ca	supplements - vit D	medication	med details	medical condition
1002	12/23/04	12/27/12	2926	8.0	1	1	1	1	0	0	0	0	0	0		
1003	01/30/02	01/03/13	3991	10.9	1	1	0	0	0	0	0	0	0	0		
1004	03/24/04	01/03/13	3207	8.8	1	1	0	0	0	0	0	0	0	0		
1006	06/05/01	01/19/13	4246	11.6	1	1	1	1	0	0	0	0	0	0		
1007	11/09/03	03/12/13	3411	9.3	1	1	1	0	0	0	0	0	0	0		
1008	08/30/01	04/13/13	4244	11.6	1	1	0		0	0	0	0	0	0		
1009	09/14/04	04/20/13	3140	8.6	1	3	0	1	0	0	0	0	0	0		
10010	07/29/01	04/20/13	4283	11.7	1	1	1	0	0	0	0	0	0	1	Adderall	1
10011	11/22/03	05/11/13	3458	9.5	1	1	0	0	0	0	0	0	0	1		
10012	02/12/03	05/11/13	3741	10.2	1	1	0	0	0	0	0	0	0	0		
10013	07/04/03	06/21/13	3640	10.0	1	1	0	1	0	0	0	0	0	0		
10014	08/19/01	06/22/13	4325	11.8	1	4	0	0	0	0	0	0	0	0		

ID	wt (kg)	ht (cm)	ht (m)	BMI	triceps	subscap	2 skin folds	body fat % - SF	Fat Free Mass	Body Fat % - BIA	Sitting_Height (cm)	Seated_Height (minus table)	Leg_length (cm)	Yrs_PHV	Tanner - P	Tanner - PH
1002	33.0	136.4	1.4	17.7	8.2	5.6	13.8	13.5	28.6	10.8	151.0	75.5	60.9	-3.88	1	1
1003	39.9	148.7	1.5	18.0	9.4	4.8	14.2	13.9	34.4	9.7	149.9	74.4	74.3	-2.60	1	1
1004	35.3	135.7	1.4	19.2	10.2	7.4	17.6	17.1	29.3	17.3	147.8	72.3	63.4	-3.74	1	1
1006	40.5	148.1	1.5	18.5	10.6	6.8	17.4	15.2	34.3	17.7	152.4	76.9	71.2	-2.05	3	3
1007	33.5	144.8	1.4	16.0	9.8	6.0	15.8	13.7	28.4	10.3	152.7	77.2	67.6	-3.14	1	1
1008	41.6	146.3	1.5	19.4	14.2	7.0	21.2	18.7	33.8	19.2	153.0	77.5	68.8	-1.97	1	1
1009	36.2	137.1	1.4	19.3	15.2	7.6	22.8	20.0	35.5	23.6	151.0	75.5	61.6	-3.57	1	1
10010	37.8	149.7	1.5	16.9	7.8	5.2	13.0	11.0	33.6	7.1	153.2	77.7	72.0	-1.97	4	4
10011	24.9	133.9	1.3	13.9	5.0	4.0	9.0	8.5	22.8	4.1	146.9	71.4	62.5	-3.70	1	1
10012	31.3	139.9	1.4	16.0	7.8	4.4	12.2	10.2	28.1	7.2	147.9	72.4	67.5	-3.20	2	2
10013	26.5	135.7	1.4	14.4	7.0	5.2	12.2	10.2	23.8	9.6	146.6	71.1	64.6	-3.50	3	2
10014	32.2	154.5	1.5	13.5	5.8	4.8	10.6	8.5	29.5	7.1	152.7	77.2	77.3	-2.06	3	3

ID	stren ex	mod ex	mild ex	activity score	PYPA - Hard Exercise	PYPA - Light Exercise	Hours spent watching TV	# of competitive activities	Activity 1	Activity 2	Activity 3	Activity 4	PYPA - Act 1	PYPA - Act 1 - h/wk	PYPA - Act 1 MET eqvt	PYPA - Act 1 - METhr/wk
1002	9	5	2	112	5	5	2	3	3	26			7	0.4	7.5	2.9
1003	4	5	3	70	5	5	3	3	15	27			11	1.0	7.3	7.2
1004	2	2	6	46	5	5	3	4	21	22	15		7	1.5	7.5	11.2
1006	7	7	6	116	2	4	4	1					11	0.7	7.3	5.4
1007	5	4	4	77	5	5	3	5	3	18	35	24	11	0.6	7.3	4.2
1008	2	6	7	69	4	5	3	3	21	17			8	0.8	4.0	3.3
1009	4	1	0	41	5	2	2	2	18				27	0.4	8.3	3.1
10010	2	3	5	48	5	5	2	4	18	17	26		11	0.5	7.3	3.6
10011	3	1	10	62	5	5	2	5	18	3	34	35	11	2.5	7.3	18.1
10012	2	3	5	48	4	3	2	3	18	21			7	0.8	7.5	6.2
10013	12	2	4	130	5	5	3	4	35	24	27		11	0.5	7.3	3.6
10014	12	0	0	108	5	5	3	3	18	37			7	0.2	7.5	1.4

ID	PYPA - Act 2	PYPA - Act 2 - h/wk	PYPA - Act 2 MET eqvt	PYPA - Act 2 - METhr/wk	PYPA - Act 3	PYPA - Act 3- h/wk	PYPA - Act 3 MET eqvt	PYPA - Act 3 - METhr/wk	PYPA - Act 4	PYPA - Act 4 - h/wk	PYPA - Act 4 MET eqvt	PYPA - Act 4 - METhr/wk	PYPA - Act 5	PYPA - Act 5 - h/wk	PYPA - Act 5 MET eqvt	PYPA - Act 5 - METhr/wk
1002	3	1.2	8.0	9.3	18	1.4	7.0	9.6	26	2.2	10.3	23.0	1.0	0.2	6.0	1.5
1003	17	0.1	6.5	0.7	7	0.2	7.5	1.2	12	0.2	3.8	0.9	9.0	2.0	6.0	11.9
1004	9	1.5	6.0	8.9	28	0.4	7.5	2.8	27	0.0	8.3	0.1	18.0	0.2	7.0	1.7
1006	29	0.7	7.3	5.4	12	0.7	3.8	2.8	9	0.8	6.0	4.6	27.0	3.5	8.3	28.8
1007	7	0.8	7.5	6.2	20	0.6	8.0	4.4	8	0.2	4.0	0.8	9.0	0.2	6.0	1.5
1008	13	0.3	7.0	2.0	33	0.1	5.0	0.6	1	0.2	6.0	1.0	9.0	1.7	6.0	9.9
1009	18	2.5	7.0	17.4	1	0.5	6.0	3.0								
10010	17	1.5	6.5	10.0	29	1.1	5.0	5.6	8	0.3	4.0	1.3	18.0	3.3	7.0	23.4
10011	17	0.4	6.5	2.7	7	1.2	7.5	8.7	20	1.0	8.0	8.3	9.0	0.7	6.0	4.0
10012	8	0.2	4.0	1.0	9	0.8	6.0	5.0	13	0.3	7.0	2.3	27.0	0.4	8.3	3.4
10013	6	0.6	5.0	3.1	17	2.5	6.5	16.1	20	0.5	8.0	4.0	8.0	2.6	4.0	10.6
10014	8	1.2	4.0	5.0	9	0.2	6.0	1.0	18	0.7	7.0	4.6	1.0	2.0	6.0	11.9

ID	PYPA - Act 6	PYPA - Act 6 - h/wk	PYPA - Act 6 MET eqvt	PYPA - Act 6 - METhr/wk	PYPA - Act 7	PYPA - Act 7 - h/wk	PYPA - Act 7 MET eqvt	PYPA - Act 7 - METhr/wk	PYPA - Act 8	PYPA - Act 8 - h/wk	PYPA - Act 8 MET eqvt	PYPA - Act 8 - METhr/wk	PYPA - Act 9	PYPA - Act 9 - h/wk	PYPA - Act 9 MET eqvt	PYPA - Act 9 - METhr/wk
1002																
1003	27	0.5	8.3	4.3	18	2.5	7.0	17.4	15	6.0	9.8	58.3				
1004	15	2.0	9.8	19.4	21	1.5	7.8	11.6								
1006	18	2.1	7.0	14.5	1	0.5	6.0	3.0								
1007	3	2.3	8.0	18.5	28	0.2	7.5	1.9	18	0.7	7.0	4.6	21	1.5	7.8	11.8
1008																
1009																
10010	22	0.8	7.3	6.0	4	0.4	3.0	1.1	26	2.0	10.3	20.4				
10011	35	0.2	9.0	2.2	18	3.7	7.0	26.0	21	0.3	7.8	2.6	1	0.5	6.0	2.7
10012	33	0.6	5.0	2.9	36	0.7	7.0	4.6	18	0.7	7.0	4.6	21	0.7	7.8	5.8
10013	27	2.5	8.3	20.6	33	0.5	5.0	2.5	18	1.0	7.0	6.9	1	1.0	6.0	6.0
10014	39	1.0	4.0	4.0												

ID	PYPA - Act 10	PYPA - Act 10 - h/wk	PYPA - Act 10 MET eqvt	PYPA - Act 10 - METhr/wk	PYPA - Act 11	PYPA - Act 11 - h/wk	PYPA - Act 11 MET eqvt	PYPA - Act 11 - METhr/wk	Total MET hrs/week	BPAQ - Current	BPAQ - Past	BPAQ - Total	Current Impact Exercise	Current Impact Exercise Frequency
1002					46.3	10.0	18.3	14.2	1	7				
1003					102.0	60.3	28.1	44.2	1	14				
1004					55.7	5.8	29.5	17.6	1	7				
1006					64.6	15.9	42.0	29.0	1	13				
1007	32	0.2	4.8	0.8	78.6	17.3	69.6	43.4	1	19	32	0.2	4.8	0.8
1008					16.8	10.5	18.3	14.4	1	2				
1009					23.4	9.7	28.8	19.2	1	6				
10010					71.6	26.6	70.2	48.4	1	14				
10011					83.2	8.6	26.7	17.6	1	12				
10012	26	1.8	10.3	18.7	56.1	17.6	65.4	41.5	1	6	26	1.8	10.3	18.7
10013	38	0.2	5.5	1.4	75.2	18.7	57.0	37.8	1	13	38	0.2	5.5	1.4
10014					27.9	23.4	28.0	25.7	1	9				

ID	Accelerometer - Sedentary	Accelerometer - Light	Accelerometer - Lifestyle	Accelerometer - Moderate	Accelerometer - Vigorous	Accelerometer - Very Vigorous	Accelerometer - Axis 1 Counts	Accelerometer - Axis 2 Counts	Cylce Ergometer WU Min#1 - HR	Cylce Ergometer WU Min#2 - HR	Cylce Ergometer WU Min#3 - HR	Cylce Ergometer WU Min#4 - HR	Cylce Ergometer WU Min#5 - HR
1002	9.0	1.1		4.3	2.3	4.4	77293	47526	173	169	181	190	202
1003	13.3	0.5		3.3	1.7	4.3	70301	34942	162	177	200	193	199
1004	10.1	1.2		5.2	1.5	3.9	68499	40540	174	189	195	204	206
1006	10.6	0.8		3.8	1.8	4.1	74433	35426	133	152	171	169	178
1007	12.3	1.2		4.9	1.7	3.9	72115	42372	148	151	156	167	178
1008	11.5	1.8		6.4	2.5	4.9	96217	54354	137	134	151	162	173
1009	6.4	1.5		6.3	1.7	4.4	78929	65378	142	167	153		
10010	8.4	1.0		2.8	0.9	4.9	81099	45095	168	166	175	180	186
10011	11.1	1.6		7.9	2.5	5.9	112467	59269	170	183	188	191	
10012	14.5	0.9		5.2	2.3	4.5	81284	59722	157	161	175	187	196
10013	5.4	1.9		5.4	2.2	5.1	88791	62383	126	139	139	150	186
10014	6.9	1.5		5.0	1.4	4.9	92651	51936	154	165	175	186	197

ID	Onset of Exercise Time	Completion of Exercise Time	Total Time (minutes)	Recovery HR	Set #1 - Drop Jumps HR	Set #1 - Lunge Jumps HR	Set #1 - Hurdle Jumps HR	Set #1 - Single-leg Hops HR	Set #1 - Jumping Jacks HR	Recovery HR
1002	9:43 AM	10:03 AM	20	130	176	179	165	192	188	128
1003	9:56 AM	10:18 AM	22	126	176	193	180	189	190	113
1004	10:02 AM	10:23 AM	21	130	189	192	188	202	198	135
1006	10:58 AM	11:18 AM	20	140	180	183	183	193	190	118
1007	9:27 AM	9:52 AM	25	81	127	144	152	167	158	113
1008	10:00 AM	10:26 AM	26	125	163	178	173	180	181	101
1009	9:19 AM	9:40 AM	21	108	165	172	170	173	159	105
10010	10:15 AM	10:31 AM	16	120	183	189	177	191	190	130
10011	10:15 AM	10:43 AM	18	135	168	170	167	181	179	135
10012	10:21 AM	10:49 AM	28	130	173	175	163	186	193	116
10013	11:37 AM	11:56 AM	19	120	158	164	170	172	177	109
10014	9:59 AM	10:19 AM	21	146	181	192	190	174	157	120

ID	Set #2 - Drop Jumps HR	Set #2 - Lunge Jumps HR	Set #2 - Hurdle Jumps HR	Set #2 - Single-leg Hops HR	Set #2 - Jumping Jacks HR	Recovery HR	Set #3 - Drop Jumps HR	Set #3 - Lunge Jumps HR	Set #3 - Hurdle Jumps HR	Set #3 - Single-leg Hops HR	Set #3 - Jumping Jacks HR
1002	170	172	176	189	181	131	173	188	178	191	193
1003	178	194	181	188	186	109	181	199	190	198	198
1004	188	183	185	192	185	131	190	194	183	190	189
1006	178	185	177	193	190	124	182	187	190	196	195
1007	152	148	141	161	162	117	149	156	153	164	157
1008	160	178	169	175	178	108	159	176	166	178	177
1009	173	187	177	173	168	120	181	192	186	178	167
10010	181	196	192	192	193	129	186	189	180	189	191
10011	168	174	170	187	183	92	164	170	172	181	181
10012	162	171	163	188	180	120	155	155	153	183	180
10013	173	159	165	181	181	123	174	158	144	177	173
10014	182	190	191	200	195	121	182	191	193	200	196

ID	dom arm	dom leg	D radius	DR z score	Dt tibia	DT z score	ND radius	NDR z score	NDt tibia	NDT z score	Total kCal Intake (kcal)	Total kCal Intake per kg (kcal/kg)
1002	R	R	3743	0	3745	1	3754	0	3738	1	2447	74
1003	R	R	3767	0	3755	1	3821	0	3709	0	1748	44
1004	R	R	3655	-1	3705	0	3599	-2	3698	0	2004	57
1006	R	R	3840	0	3790	1	3889	1	3758	1	1240	31
1007	R	R	3801	0	3857	2	3909	1	3805	2	2065	62
1008	R	R	3726	-1	3737	1	3726	-1	3705	0	1560	37
1009	R	R	3854	1	3694	0	4023	2	3691	0	2620	72
10010	R	R	3775	0	3594	-1	3766	0	3613	-1	2069	55
10011	R	R	3720	-1	3625	-1	3751	0	3693	0	2093	84
10012	R	R	3699	-1	3621	-1	3640	-2	3647	0	1513	48
10013	R	R	3879	1	3757	1	3825	0	3835	2	1581	60
10014	L	L	3809	1	3653	1	3831	1	3584	0	1718	53

ID	Total Energy - CHO (g)	Total Energy - FAT (g)	Total Energy - PROTEIN (g)	Total Calcium 24Hr (mg)	Total Vitamin D (ug)	Total Vitamin D (IU)	Total Sodium (mg)	Total Potassium (mg)	Total Phosphorus (mg)	Total Magnesium (mg)	Total Zinc (mg)	Total Caffeine (mg)
1002	297	95	106	650	5	169	3231	2672	1208	282	8	0
1003	262	54	62	1075	6	258	2535	2219	1108	266	14	3
1004	299	62	76	1319	7	307	2936	2952	1304	358	11	1
1006	177	46	37	655	2	63	1853	843	339	79	2	0
1007	286	72	79	1195	7	295	3170	1719	1294	214	10	2
1008	294	22	53	426	1	58	1518	2358	707	180	4	0
1009	366	95	83	858	4	115	2558	2630	952	217	6	0
10010	227	99	76	1314	13	421	2607	3251	1654	301	10	1
10011	392	35	79	1099	5	185	1468	4901	1489	421	10	1
10012	261	32	53	897	7	308	1880	3227	1029	283	6	2
10013	222	57	55	429	1	36	2474	1131	707	167	6	2
10014	293	35	63	824	5	187	1555	2256	1014	238	6	0

ID	NTX - T1 (nM)	Dilution Factor 1	NTX - T2 (nM)	Dilution Factor 2	NTX - T3 (nM)	Dilution Factor 3	NTX - T4 (nM)	Dilution Factor 4	BAP - T1 (µg/L)	Dilution Factor 1	BAP - T2 (µg/L)	Dilution Factor 2
1002	20.53	61.60	25.28	75.85	19.65	58.94	22.43	67.29	15.55	46.66	19.95	59.85
1003	23.23	69.69	23.67	71.02	17.50	52.49	14.46	43.37	38.81	116.43	42.77	128.31
1004	12.08	36.25	15.26	45.78		64.23	14.11	42.34	42.59	127.77	40.48	121.45
1006	22.12	66.35	16.92	50.76	24.35	73.05	29.92	89.75	71.18	111.90	65.48	105.12
1007	18.13	54.38	26.68	80.05	19.98	59.93	16.20	48.60	25.16	75.49	21.99	65.98
1008	13.50	40.49	26.27	78.81		64.23		58.58	34.37	103.12	22.63	67.88
1009	14.77	44.32	14.94	44.82	18.56	55.68	11.92	35.76	50.03	150.08	52.11	156.33
10010	20.13	60.40	19.52	58.55	19.36	58.07	21.56	64.68	40.07	120.22	39.78	119.33
10011	8.40	25.20	13.30	39.90	13.43	40.29	16.25	48.75	47.11	141.32	49.45	148.34
10012	14.72	44.16	4.86	14.58	26.46	79.38	19.70	59.10	43.64	130.91	19.17	57.52
10013	14.92	44.77	37.55	112.64	33.41	100.22	28.72	86.17	30.36	91.09	32.60	97.80
10014	52.61	49.78	16.89	61.16	19.86	64.23	18.26	58.58	42.61	127.82	44.52	133.57

ID	BAP - T3 (µg/L)	Dilution Factor 3	BAP - T4 (µg/L)	Dilution Factor 4	OPG - T1 (pmol/L)	OPG - T2 (pmol/L)	OPG - T3 (pmol/L)	OPG - T4 (pmol/L)
1002	27.17	81.51	36.02	108.05	3.05	2.97	2.67	3.22
1003	47.68	143.05	41.48	124.43	2.21	2.65	2.28	1.82
1004		116.55	41.69	125.06	3.92	3.39	4.21	3.08
1006	60.00	116.55	66.48	131.95	3.61	3.79	4.10	3.89
1007	22.48	67.44	25.77	77.31	3.38	4.08	3.87	4.31
1008		116.55		131.95	2.73	3.59	4.21	3.90
1009	54.43	163.28	56.79	170.37	5.11	6.18	5.33	5.37
10010	10.20	30.60	35.86	107.59	3.26	4.14	4.42	3.75
10011	52.80	158.39	60.34	181.02	5.25	5.86	6.55	5.83
10012	59.68	179.04	58.14	174.43	4.05	5.69	4.75	4.85
10013	37.01	111.04	45.69	137.06	3.99	3.68	4.59	4.08
10014	38.19	114.56	38.05	114.14	3.77	3.44	3.53	2.64

ID	RANKL - T1 (pmol/L)	Dilution Factor 1	RANKL - T2 (pmol/L)	Dilution Factor 2	RANKL - T3 (pmol/L)	Dilution Factor 3	RANKL - T4 (pmol/L)	Dilution Factor 4
1002	5.45	544.65	6.21	620.88	5.71	571.45	5.60	560.12
1003	4.32	432.41	3.32	331.73	3.72	372.31	4.41	440.78
1004	4.36	435.76	3.27	327.37		323.02	3.39	338.69
1006	1.72	172.45	1.88	187.70	1.24	123.60	1.54	154.11
1007	8.57	317.43	6.75	317.58	7.78	323.02	7.32	340.71
1008	2.88	287.70	2.24	224.21		323.02		340.71
1009	1.06	105.52	1.41	141.41	1.94	194.32	1.50	150.21
10010	4.35	434.93	3.85	385.12	4.72	471.56	5.90	590.15
10011	4.69	469.07	5.00	500.44	4.67	466.58	4.44	444.12
10012	0.70	70.29	1.23	122.76	1.19	118.66	0.94	93.54
10013	0.49	49.13	0.61	61.33	0.37	36.65	0.41	41.37
10014	4.90	489.78	5.90	590.39	5.52	552.06	5.94	594.00